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RICHARD KENNETH BRUMMITT: 1937–2013



It is with sad hearts that we dedicate this series of papers on paraphyly to our late friend and colleague Dick Brummitt, who passed away on 18 September 2013. Due to declining health, he was unable to travel to Melbourne for presentation of his talk at the IBC symposium in 2011, but he did prepare a video of his lecture that was shown during his time slot on the program (still available online at: <http://www.uni-goettingen.de/downloads/Dick_Brummitt_Conference_video.mp4>). Although this video format did not allow questions, it nonetheless was effective in stimulating discussion from the audience. Dick was a person who could be passionate regarding particular ideas, and the necessity of paraphyly in biological classification was one of these. His feelings came through clearly and forcefully in his oral presentation. We are proud and honored to have his paper included here in this symposium series. We wish he were still with us to continue the discussions.

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EVOLUTIONARY SYSTEMATICS AND PARAPHYLY: INTRODUCTION[1]

Tod F. Stuessy[2] *and Elvira Hörandl*[3]

ABSTRACT

Phylogenetic systematics, especially involving molecular data, has had a remarkable impact on systematic biology. Numerous tree-building computer programs exist for the reconstruction of phylogenies, and many packages are available for analysis of population genetic data for estimating genetic divergence within and among populations. These advances have come about through the joining of statistical algorithms, computer programs, and DNA base-pair sequence and fragment data. Deeper genomic data are on the horizon for use with similar questions, and the next several years will witness many spectacular genetic advances. While great progress is being made on analytical approaches with molecular data in systematics, the use of the results of these analyses in biological classification has solidified into a dogmatic view, which has impeded further progress. Emphasis still remains on using only synapomorphies, even single characters, for delimitation of groups, on insisting that sister groups should have the same rank, and admitting only holophyletic (= monophyletic s. str.) groups. Evolutionary divergence within lineages and reticulate evolution are often ignored. As a result of these processes, paraphyletic groups, i.e., monophyletic groups that do not contain all descendants from a common ancestor, are often rejected. Evolutionary systematics takes the processes of descent and modification into consideration for reconstructing phylogenetic relationships involving many dimensions. This symposium presents various approaches for recognizing cladogenetic, anagenetic, and reticulate evolution in different organisms, which help reveal micro- and macro-evolutionary processes. Controversy still exists regarding how taxonomists should incorporate the diversity of evolutionary patterns and processes into biological classification. Case studies demonstrate that purely phylogenetic (cladistic) concepts of classification are unsatisfactory in cases of non-hierarchical relationships. Contributions also deal with the controversial question of recognition of paraphyletic groups in classification.

Key words: Cladistics, classification, divergence, evolution, patristics.

Biological classification has a complex history. In pre-Linnaean and Linnaean times, classification was relatively simple, in that one sought differentiating characters that were chosen by God to distinguish all created organisms. The development of natural systems of classification in the 18th century emphasized the importance of using constellations of characters together for maximum predictive quality of classifications. In the 19th century, Darwin (1859) taught that groups established through natural classification have derived by descent and modification from common ancestors. This perspective served as a basis for evolutionary classification until appearance of quantitative approaches in the last half of the 20th century.

Beginning in the late 1950s, dissatisfaction with intuitive approaches to science, including classification, led to development of more precise methods. Discovery of the molecular structure of DNA (Watson & Crick, 1953), the basis of heredity, suggested that many biological phenomena might be interpretable in quantitative terms. Phenetics, or the assessment of overall similarity using numerical methods, developed as the first attempt at quantitative classification (Michener & Sokal, 1957; Sneath, 1957; Sokal & Sneath, 1963). To achieve this end, evolutionary assumptions were minimized, which initially proved to be intellectually stimulating, but later was regarded as insufficient, as basic realities of the organic world were ignored. Into this challenge came cladistics (Hennig, 1966), which attempted to reconstruct branching patterns of evolution (or phylogeny). To successfully provide a means of ease of calculation and convenient inter-conversion between diagram (cladogram) and resultant classification, cladistics ignored divergence within lineages. Despite this limitation, cladistic analysis has become an important tool of evolutionary analysis in many areas of biology, along with the re-employment of phenetic algorithms that are more efficient when analyzing large sets of molecular data.

[1] This and the following seven articles are the proceedings of the "Evolutionary Systematics and Paraphyly" symposium held during the International Botanical Congress, Melbourne, Australia, 28 July 2011.

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During developments of phylogenetic analysis over the past 30 years, however, a number of workers have suggested that despite positive contributions, cladistic *classification* is too narrow because it does not reflect full dimensions of phylogeny. Quantitative evolutionary classification, therefore, developed in the 1980s. This involved adding quantitative values to character divergence within lineages, to be combined with the dichotomous patterns of relationship determined via cladistic analysis. In a sense this represented returning to evolutionary classification prior to phenetics and cladistics, but now also in a completely quantitative framework. Several approaches have been developed, such as convex phenetics (Estabrook, 1986), addition of cladistic and patristic distances in a single data matrix, followed by appropriate clustering (Stuessy, 1987), patrocladistics (Stuessy & König, 2006), etc. Although these methods for quantitative evolutionary classification have not received widespread use, they have been sufficient to indicate that other aspects of phylogeny can, indeed, be incorporated quantitatively into biological classification. That is, there is certainly no need to be limited only by branching pattern information. If representation of phylogeny is really the goal of biological classification, then we should aggressively seek quantitative means of obtaining more, rather than be satisfied with fewer, dimensions of phylogeny.

The Meaning of Paraphyly

Paraphyly is not a contagious disease. It is also not an evolutionary grade. Paraphyly is an evolutionary stage of monophyletic taxa (s.l.) that needs to be taken into account for purposes of biological classification. Arguments for this perspective and suggestions on how this should be done successfully have formed the principal focus of the present symposium.

The main area of conflict between cladistic and evolutionary classification is the insistence of the former on strict holophyly. A holophyletic group is one that includes the immediate ancestor and *all* its descendents, independent of whatever divergence occurs within each of the derivative lineages (Ashlock, 1971). A paraphyletic group is one that derives from a common ancestor but that does not contain all its descendents (Hennig, 1966). For example, a new species that buds off from a parental set of populations forms a new holophyletic species, but the progenitor then becomes paraphyletic, and hence, unacceptable following cladistic conventions.

After Darwin (1859), all groups in classification were regarded as being evolutionary units, having been derived from the evolutionary process. All legitimate groups in classification (i.e., the taxa placed into classes of the hierarchy) were monophyletic. Non-monophyletic groups, that is, those that are polyphyletic, deriving from two or more independent ancestors, were unacceptable, then as now. This perspective endured successfully until the rise of cladistics in the 1960s, which coincides most conspicuously with the appearance of the English version of Hennig's book (1966). To provide a *convenience* of transformation of a branching pattern into a hierarchical classification and the reverse, that is, to have no ambiguity, Hennig narrowed the concept of monophyly to refer to an ancestor and all its descendents. This new definition, however, excluded groups that derive from a common ancestor but that do not include all the descendents. Hennig named these latter groups paraphyletic and excluded them from acceptable classification. Because this created a significant deviation from previous perspectives, Ashlock (1971) recommended calling this more narrow perspective holophyly and regarded both it and paraphyly as forms of monophyly s.l. We advocate this perspective (Hörandl & Stuessy, 2010; Stuessy & Hörandl, 2014), which is also maintained in many papers of this present symposium.

Paraphyly as Part of the Evolutionary Process

Paraphyly, therefore, is very much a part of the evolutionary process (Hörandl, 2007). Essentially, paraphyly and holophyly are alternating conditions that cycle forward and change over many generations (Hörandl, 2006; Hörandl & Stuessy, 2010). A newly originated species usually is holophyletic (= monophyletic s. str.), but depending upon its mode of origin, it can leave the progenitor holophyletic or paraphyletic. With geographic allopatric speciation, two sets of populations diverge equally (or nearly so) from each other, resulting in extinction of the progenitor in the process. In a pattern sense, the entire group remains holophyletic because divergence is symmetrical. Another case would be adaptive radiation, common in oceanic islands, whereby divergence in many different geographical and ecological directions occurs simultaneously, all equidistant in divergence from the progenitor, which in the process ceases to exist. Conditions in which the progenitor is rendered paraphyletic, whereby divergence is asymmetrical, occur with several types of progenitor-derivative speciation (Crawford, 2010). One common example involves budding of new populations that continue to accrue reproductive isolation and speciation, leaving the parental populations virtually intact (Hörandl & Stuessy, 2010). As

emphasized by Stuessy et al. (2014), this type of progenitor-derivative speciation exists within oceanic islands, whereby colonizers from large genera disperse successfully to a new island environment and there evolve into one or more groups of new distinctive endemic species, to such an extent that they may be regarded as distinct genera. Another type of evolution that results in paraphyletic parental taxa is reticulate speciation, i.e., allopolyploidy, whereby both parents involved with the initial hybridization become paraphyletic after formation of the successful daughter polyploid. Several reviews have estimated high levels of paraphyly at the specific level (e.g., Rieseberg & Brouillet, 1994; Funk & Omland, 2003).

Objectives of the Symposium

Interest in aspects of cladistics, evolutionary systematics, and paraphyly led to the holding of a symposium on the topic "Evolutionary systematics and paraphyly" during the International Botanical Congress, Melbourne, Australia, 28 July 2011. The objective was to present different perspectives toward moving forward from simple cladistic classifications to models that contain more phylogenetic information, all in a quantitative framework.

It is very appropriate at this point in time to consider seriously these issues. One segment of our systematics community is already convinced that phylogenetic analysis, using mainly molecular data, with interpretation using cladistic rules of classification, is the solid way for information structuring now and into the foreseeable future. Other workers in our community, however, are very supportive of phylogenetic analysis using molecular data, but find that strict cladistic classification, with reliance on holophyly, is not the appropriate information structure to serve societal needs either now or certainly not into the future. More progress needs to be achieved, as has always been the case in the development of our field. As one example, some workers are highly supportive of the Angiosperm Phylogeny Group (APG) classification of angiosperms (APG, 1998, 2003, 2009), based largely on DNA data and phylogenetic analysis, *and* interpreted in the context of cladistic rules of classification. Although this initiative, in general, represents a clear advance in phylogenetic synthesis for the entire botanical community, some of the results presented distorted information content regarding certain families. Plantaginaceae, for example, now contain genera with virtually no morphological resemblance to the genus *Plantago* L., and not surprisingly, some workers find it difficult to accept this approach (Brummitt, 2014).

The papers in this symposium reflect different interests in evolutionary systematics, with investigations focusing on the nature of species at the populational level (Lockhart et al., 2014), the problem of holophyly and paraphyly and conservation impact on recognition of endemic genera in oceanic islands (Stuessy et al., 2014), the utility of paraphyly in addressing specific and generic concepts (Ehrendorfer et al., 2014; George, 2014; Hörandl, 2014), methods for finding support values for phylogenetic lineages (Zander, 2014), and issues relating to holophyly and paraphyly among angiosperm (dicot) families (Brummitt, 2014).

We recognize that this one symposium will not solve all issues relating to biological classification. We strongly believe, however, that the time has come to look critically at simple cladistic classification couched in a narrow evolutionary context and expand this into quantitative evolutionary approaches that capture more phylogenetic information. This is the only way to provide a more information-rich system with higher predictive value for biology and society as a whole. This was the spirit of the symposium.

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WE ARE STILL LEARNING ABOUT THE NATURE OF SPECIES AND THEIR EVOLUTIONARY RELATIONSHIPS[1]

Peter J. Lockhart,[2] Anthony W. D. Larkum,[3] Matthias Becker,[2] and David Penny[2]

ABSTRACT

Early evolutionary thinkers proposed relatively simple models to describe processes of evolution, and these are the basis of evolutionary models still used today. Recent research has since shown that evolutionary relationships among plants can be complex and difficult to reconstruct even from molecular data. In plants there is a continuum of processes, ranging from reticulate relationships within a sexually reproducing population, incomplete lineage sorting and hybridization between recently diverged species, allopolyploidy between more distantly related species, to symbioses and endosymbiosis. These aspects of plant biology can create practical problems for interpreting bifurcating gene trees and identifying species. The promise of "omics" is that it will provide data and analyses to improve our understanding of the nature of species and their phylogenetic relationships. We highlight the importance of distinguishing evolutionary processes and evolutionary models, and stress that improving the understanding of micro-evolutionary processes is necessary to inform current debate on whether or not to accept paraphyletic species.

Key words: Evolution, genomics, hybridization, paraphyly, species concepts.

Most researchers agree that practicality, usefulness, and predictability are important considerations for whether or not paraphyletic species should be recognized in taxonomy. However, while some authors suggest that biological classification should capture more evolutionary information (Hörandl & Stuessy, 2010), others question whether the acceptance of paraphyletic species as units for classification better reflects processes of evolution and patterns of biological diversity (Schmidt-Lebuhn, 2011). A central question to resolve is: *What can phylogenetic analyses tell us about the nature of species and evolutionary relationships*? This question, and the debate over recognition of paraphyletic species, is rapidly being informed by genome science. Its promise is that it has the potential to tell us much about paraphyly, polyphyly, repeatability of evolution, and the spatial and temporal extent of interspecific gene flow. This information is important for understanding the nature of species and the suitability of gene tree and species tree methods for reconstructing evolutionary histories and developing robust taxonomies.

THE NOTION OF SPECIES USED WHEN BUILDING SPECIES TREES

It has been said that "all that is empirically demonstrable are species—they must exist because we observe discontinuities between groups of organisms (Coyne & Orr, 2004)—and clades, which are the inevitable consequence of common ancestry and speciation processes (Wiley, 2009), and are recognizable from their synapomorphies" (Schmidt-Lebuhn, 2011: 178).

The idea of "species" as a fundamental unit certainly predates Darwin and Wallace. Earlier concepts were not of species but were "forms" and "kinds" of plants and animals that could even appear spontaneously (as mentioned in the King James Version of the Bible published in 1611). However, during the 17th century the concepts of "species" and "genera" developed, and as far as we can tell, John Ray, in his Natural Theology book of 1691, was one of the earliest writers to discuss species in a modern sense (Raven, 1986). As pointed out by Mayr (1968: 165), Ray, in discussing criteria that identify species, considered it essential that species "spring from the seed of one and the same . . ." This is an idea in essence equivalent to the biological species concept that would obtain its popular form 250 years later. Ray gathered plants from various places in England and grew them all in his own garden, thus, separating inherited and environmental effects. He concluded that "varieties" could come from the same parent plant and were members of the same species.

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These early concepts of having "members" of a set (species) within larger sets (genus) were also used by other authors at the time to describe different kinds of crystal structures (species) in rock. In doing so, there was no suggestion or implication that species, whether plants, animals, or rocks, could ever change or merge into other species over time. This way of thinking was formalized in the mid-18th century when Linnaeus (1736) codified a system that was universally adopted for classifying animals and plants, and which had as its basic unit the species. Much later, when championing the biological species concept, Mayr also emphasized the discrete and distinct nature of species—a consequence of individuals from one species being reproductively isolated from individuals of another species (Mayr, 1963; Donoghue, 1985). Although some botanists have abandoned the biological species concept when discussing plant diversity, it is Mayr's way of thinking about species that has most influenced the development of modern methods for species delimitation (Fujita et al., 2012) and current methods for species tree reconstruction. An important question is whether such approaches are generally appropriate in plant systematics.

Adoption of a Treelike Model for Evolution

The developers of species tree methodology have taken some ideas from Darwin but not others. Darwin was convinced of descent by modification and at least partly by the treelike nature of evolutionary processes (Penny, 2011). He recognized morphological discontinuities as the result of the process of divergence and extinction (Mallet, 2008a). One could assume that it was a species tree that he had in mind when he first sketched a sticklike tree figure in his now famous notebook (see <http://darwin-online.org.uk/>), but it could also have been populations or varieties. It is not clear what he meant in terms of evolutionary relationships and the biological processes that were depicted. Both Darwin and Wallace had a notion for species trees and models for describing evolutionary relationships between species. In his 1855 paper, Wallace referred to "trees" and "evolution" in at least two places. In the first, he says "Much discussion has of late years taken place on the question, whether the succession of life upon the globe has been from a lower to a higher degree of organization? (p. 191)... and the progression from Fishes to Reptiles and Mammalia, and also from the lower mammals to the higher, is indisputable In the second passage that immediately follows, he says, "returning to the analogy of a branching tree, as best mode of representing the natural arrangement of species and their successive creation, let us suppose that at an early geological epoch any group (say a class of Mollusca) has attained to great richness of species and high organization. Now let this great branch of allied species by geological mutations, be completely or partially destroyed. Subsequently a new branch springs from the same trunk, that is to say, new species are successively created, having their antitypes the same lower organised species which had served as the antitypes for the former group, but which have survived the modified conditions, which destroyed it. ..." It is clear that Wallace had the idea of a phylogenetic tree (and Darwin noted that he "uses my simile of tree" on his copy of Wallace's paper, probably annotated about 1856–1857), even though Wallace had not fleshed out this concept in detail (Brooks, 1984).

In *On the Origin of Species*, Darwin (1859) goes over much of the same ground as Wallace. One might think that it is a species tree, but he, too, does not detail the nature of this tree; the only tree figure in *On the Origin of Species* is one that Darwin largely moved from his unpublished "Big Book" (Stauffer, 1975), where Darwin had another purpose in mind for this tree. There it was bound up with his somewhat undefined concept of "The Principle of Divergence." Simply put, "The Principle of Divergence" is the outcome of the "Struggle for Existence" in terms of species (or as the full title to *On the Origin of Species* puts it "By Natural Selection or the Survival of Favoured Races in the Struggle for Life"). However, note that neither Wallace nor Darwin followed this through in a tree that mapped out how a species tree might look. Darwin's (non-binary) tree as first envisaged in the "Big Book" showed how populations, varieties, and species might change under competition and under the forces of extinction (not individually but over generations), and it was not concerned with a genealogy showing all species formations and losses. In *On the Origin of Species*, Darwin used much the same diagram to map how a group of related organisms (species or subspecies?) might change over time. However, in the diagram, Darwin did not define species. He was very careful not to do this, and he viewed the situation as being very plastic between races, species, and genera.

Perhaps, ironically, it is Darwin's treelike representation that is the evolutionary model adopted for reconstructing and visualizing evolutionary relationships, while at the same time he has been criticized for his concept of species (Mallet, 2008a). This duality also applies to recent species tree reconstruction methods, which assume an evolutionary treelike model but do not model gene flow between species.

Building Species Trees

Recent years have seen exciting developments in methodology for reconstructing evolutionary relationships between species. In particular, based on the assumption that species comprise monophyletic assemblages of individuals, numerous new "multi-species coalescent" methods have been developed for inferring evolutionary relationships from nucleotide sequence data (Knowles, 2009; Kubatko et al., 2009; Liu et al., 2009; Heled & Drummond, 2010; Fan & Kubatko, 2011; Bryant et al., 2012). These methods assume a treelike model for evolution and accept that, while individual gene trees on occasion might indicate paraphyly, such gene trees have, nevertheless, evolved on an underlying species tree in which each species is genetically isolated; thus, individuals of each new species are monophyletic with respect to individuals of other species. Species tree building methods do not test whether or not this is an appropriate model for describing the evolutionary relationships among the taxa being studied.

Tests for inferring the evolutionary significance of observed patterns of monophyly among gene trees can be applied prior to species tree reconstruction and identification of reproductively isolated "species" based on minimum expectations for the extent of monophyly observed in analyses of independent genes (e.g., Rosenberg, 2007). Similarly, since the expected theoretical distribution of gene trees on a given species tree can be calculated (Degnan & Salter, 2005), then given a large enough sample of independent gene trees, in principle it is possible to examine whether there is a good fit between the optimal species tree (representing the evolutionary model) and the observed gene trees (Fan & Kubatko, 2011). In practice, this is not done. However, as we discuss below, it is possible to test whether or not gene trees have evolved under evolutionary models involving hybridization. In this case, if statistical evidence rejects a simpler evolutionary scenario, it is more difficult to reconstruct multi-species coalescent species trees.

Despite this concern, multi-species coalescent methodology has value for plant systematics because radiations have been important in the formation of many extant plant species (e.g., Martin et al., 2005; Winkworth et al., 2005; Linder, 2008; Pennington et al., 2010; Valente et al., 2010). Under such evolutionary scenarios, reconstructed gene trees are likely to contain short internal branches and long external branches. This is a situation in which phylogenetic accuracy can be low, particularly where there is substitution model misspecification (Hendy & Penny, 1989; Shavit et al., 2007). Furthermore, even if accurately determined, gene trees can be discordant with the true underlying species tree. This is a theoretical expectation where ancestral population sizes of extinct species are relatively large and where times between diversification events are relatively small at the base of the species tree. Indeed, there are certain combinations of ancestral population sizes and divergence times where the majority of gene trees are expected to be incongruent with the species tree on which they have evolved (Degnan & Rosenberg, 2009). For these reasons, the idea of a methodology for reconstructing species relationships that accounts for the problem of discordant and paraphyletic gene trees due to incomplete lineage sorting is very appealing. Thus, the hope is for more reliable phylogenetic inferences from multi-species coalescent methods that could be used by biologists to inform taxonomy.

The problem is that the multi-species coalescent models currently used for building species trees do not model gene flow between species, and for organisms that diverge with gene flow, it is unclear how effective the methodology is. In practice, too few empirical studies have been undertaken to yet know whether or not current implementations of multi-species coalescent tree methods provide a general and useful tool for plant systematics and taxonomy. One recent study examined six species of wild rice and noted extensive discordance of gene trees for these species. The authors reported more discordance than was expected under a coalescent model with no gene flow. Not surprisingly the authors found that a Bayesian species tree method (BEST) was unable to converge on the expected species tree topology (Cranston et al., 2009). Recent work has also used computer simulations to study the performance of the same BEST and the impact of horizontal gene transfer (as might occur through interspecific hybridization). In this case, it was found that when introgressed sequences were distributed asymmetrically between species (meaning a greater proportion of the genome of species A was present in species B than the genome of B was in species A), BEST also performed poorly (Chung & Ané, 2011). Nevertheless, this is still not very much information to go on, and the disappointing result might reflect more the coalescent model used rather than the method itself. There are coalescent models that do not assume symmetric patterns of gene flow (e.g., such as that developed by Beerli & Felsenstein, 2001), and while these are not yet implemented in building species trees, perhaps such models represent a useful direction for future research.

Although hybridization between some species has gained only recent acceptance in the zoological research community (e.g., see discussions by Mallet, 2007, 2008b), almost 300 years of botanical investigation suggest its importance for understanding the nature of species and describing their relationships (e.g., Ehrendorfer, 1959; Stebbins, 1959; Arnold, 1997; Rieseberg, 1997). Recent work recognizes the occurrence of hybridization in both animal and plant species radiations (Herder et al., 2006; Mallet, 2007; Soltis & Soltis, 2009; Stemshorn et al., 2011) and in evolutionary adaptation to environmental change (Hoffman & Sgro, 2011). If hybridization is as common and evolutionarily significant as many researchers have now suggested, then multi-coalescent species tree methods in their current form might have limited applicability for reconstructing plant species relationships and informing debate on recognition of paraphyletic species.

New Zealand Alpine *Ranunculus*

Hybridization is regarded as a conspicuous feature of the New Zealand flora (Cockayne & Allan, 1926). Within this flora, a group of 20 or so alpine *Ranunculus* species was described (Fisher, 1965; Webb et al., 1988; Heenan et al., 2006) as an adaptive radiation wherein convergent morphologies appeared in similar habitats across the New Zealand landscape. Phylogenetic analyses of nuclear ITS and chloroplast *ycf*1 sequences from New Zealand alpine *Ranunculus* species reported in Lockhart et al. (2001) uncovered numerous examples of non-monophyletic relationships in reconstructed gene trees. Additional sequencing of the same loci has since identified further examples of non-monophyly. Figure 1 illustrates relationships inferred for species representing one of the two main breeding groups. It has been unclear to us how such patterns of non-monophyly should be interpreted. Different species from the same geographic regions have nuclear ITS and chloroplast *ycf*1 sequences more similar to those of other species from the same geographic locality than they do to members of their "own" species. Thus, the question for us is similar to that posed at the beginning of this article: *What are the gene tree analyses telling us about the nature of New Zealand alpine* Ranunculus *species and their evolutionary relationships*?

We speculate that these species might be explained by Mallet's (2007) genotypic cluster species concept. That is, while ecologically and morphologically significant traits and their underlying genetic determinants delimit these species, there has, nevertheless, been regional gene flow of neutral genetic markers between sympatric reproductively compatible species. This interpretation would also be consistent with Fisher (1965) in that both interspecific hybridization and divergence are necessary to explain morphological variation within and between the natural populations of the species he described.

We are currently collecting more genetic data to test this hypothesis using the analytical approaches described in the following section. If our hypothesis is correct, it will have implications for using multi-species coalescent tree reconstruction methods with our data. Most notably, if we were to include an increasing number of gene trees for neutral gene loci in a species tree reconstruction for New Zealand alpine *Ranunculus* given possibly high levels of regional interspecific gene flow, we would not expect reconciliation of discordant gene trees in a way that would represent a meaningful species tree. Given this possible concern, a first step in improving our understanding is to identify the occurrence and extent of past hybridization events involving alpine *Ranunculus* species. Until recently, and despite a general consensus for the importance of hybridization in plant evolution, quantifying the extent of natural hybridization has been a difficult issue to investigate in a rigorous manner (Arnold, 1997; Brumfield et al., 2008; Joly et al., 2009).

Distinguishing Hybridization from Lineage Sorting

New methods based on coalescent models have recently been proposed for evaluating introgression (e.g., Joly et al., 2009; Pelser et al., 2010; Gerard et al., 2011; Joly, 2012). They might be applied after first noticing discordance between gene trees, paraphyly, or polyphyly in some reconstructed gene trees but not in others. In the first method, a species tree is inferred from independent gene trees whose genes in the taxa studied are assumed to be unaffected by hybridization. Gene trees are then simulated on this species tree (or posterior distribution for the species tree) assuming a coalescent model that allows for incomplete lineage sorting but not hybridization. The genetic distances between taxa in these simulated gene trees are then compared against genetic distances in the gene trees reconstructed for taxa and molecular markers which are being evaluated for evidence of introgression. Specifically, in instances where non-monophyly is observed in reconstructed gene trees, the question is asked whether genetic distances in the observed gene trees are significantly less than those expected in computer simulations under an assumed coalescent model. If so, then lineage sorting is excluded as an explanation for the

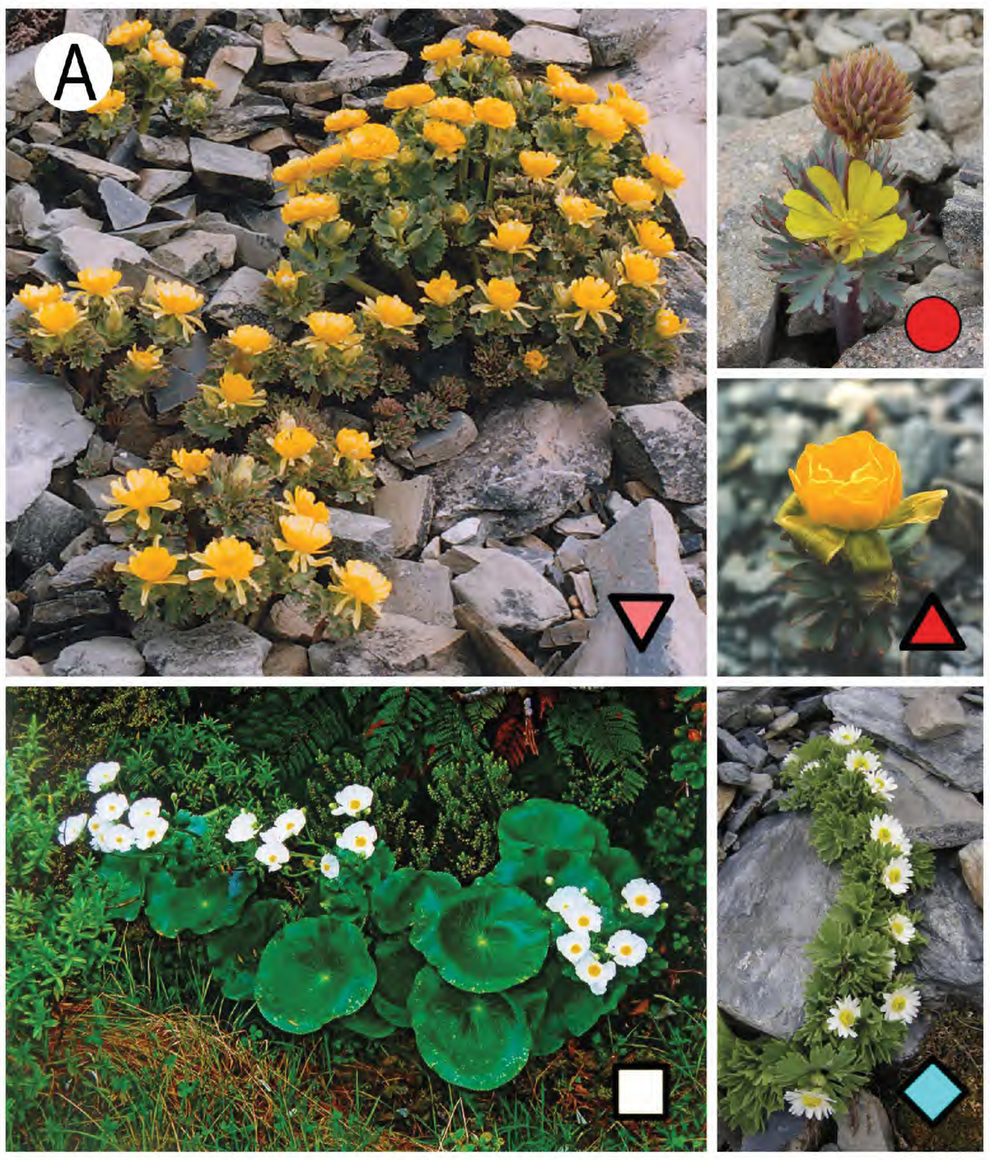


Figure 1. —A. New Zealand alpine *Ranunculus* species and microhabitats, photo *R. acraeus*: Anne Cartman, photo *R. buchananii*: Bruce van Brunt. —B. Geographic distributions according to Lockhart et al. (2001) and Heenan et al. (2006). —C, D. Accessions sequenced for C and D are numbered: 1, Borland; 2, Mt. Anglem; 3, Edwards; 4, Jagged Stream; 5, Mt. Franklin; 6, Temple Basin; 7, Canyon Creek; 8, Franz Josef; 9, Hooker Valley; 10, Mt. Cook; 11, Mark Range; 12, Mt. Earnslaw; 13, French Ridge; 14, Lake Harris; 15, Mt. Tutoko; 16, Ocean Peak; 17, Mitre Peak; 18, Amuri; 19, Lake Tekapo; 20, Mt. Hutt; 21, Mt. St. Patrick; 22, Ben Ohau; 23, East Dome; 24, Eyre Mts.; 25, Symmetry Peaks; 26, Mt. Pisgah; 27, St. Mary; 28, Homer; 29, Lake Alta; 30, Mt. Brewster; 31, Mt. Burns; 32, Mt. Earnslaw; 33, Skeleton Lake; 34, Takitimu Mts. —C. Neighbor joining tree of chloroplast *ycf*1. —D Neighbor joining tree of nr ITS sequences. In Lockhart et al. (2001), the *ycf*1 region was referred to as JSA—the region where *ycf*1 is located in the chloroplast genome. Trees were constructed with Geneious version 5.6 (<http://www.geneious.com/>).

non-monophyly, and hybridization is inferred. If the distances are not significantly less, then incomplete lineage sorting remains a possible explanation for the data. The approach suggested by Pelser et al. (2010) is similar, but these authors invert the argument. Certain genetic distances between different species are only expected given certain ancestral population sizes. If estimates of these population sizes are far greater than is reasonable, then incomplete lineage sorting can also be rejected. In Meng and Kubatko (2009) and Gerard et al. (2011), an estimate of the extent of introgression in an a priori specified taxon is made by comparing observed gene trees with trees simulated on the parental trees (or "principal" trees

as they have been called in Holland et al., 2008) embedded within a rooted reticulate phylogenetic network.

All of the above methods require that the reference species tree (or posterior distribution of species trees: Joly, 2012) is reliably inferred and not itself impacted by hybridization. While this is a limitation (and potentially can introduce circularity), in practice it means that implementations of the test need to be conservative, and simulations might not be able to be conducted using all species belonging to a group. When the test of Joly et al. (2009) was proposed, we illustrated the test using a data set similar to that shown in Figure 1. However, at $P = 0.05$ level we could not reject lineage sorting as an explanation for the observed patterns of paraphyly. We felt this might be the result of the relatively short concatenated chloroplast sequences used in the analyses. We have now sequenced complete chloroplast genomes for some of our alpine *Ranunculus* species (unpublished) and also made progress in developing EST libraries for alpine *Ranunculus* species using Illumina sequencing protocols (Atherton et al., 2010; Gruenheit et al., 2012). However, the analyses from this further work are not complete at the time of writing. The hope is that, given these additional data, we will soon be in a better position to understand the meaning of the paraphyletic gene trees observed in phylogenetic reconstructions of our species (Fig. 1). That is, we look to these data to help us determine what gene trees are telling us about the nature of our species and their evolutionary relationships.

Genome Science and the Nature of Species

Recent studies have provided an overwhelming number of species concepts (see <http://www.ucl.ac.uk/taxome/jim/Sp/species.pdf>) and a sense that authors are often referring to different entities when discussing species in their favorite group of organisms. Introgression and horizontal gene transfer between species have evidently occurred in many groups of organisms—plants, animals, and microbes (Dagan et al., 2008; Mallet, 2008b). These inferences have been made as a result of the increasing number of genetic markers that have become available for non-model organisms. With methods such as those described above, the power for detection of hybrids increases greatly (Joly et al., 2009; Gerard et al., 2011). It is exciting to think that very soon, with high-throughput sequencing of genomes and transcriptomes, we will have our best glimpse yet of the genetic distinctiveness of species, their evolutionary transience, and complexity of relationships.

Conclusions

The significance of paraphyletic taxa in our phylogenetic reconstructions will become clear once we have a better understanding of the nature of species and their evolutionary relationship with other species. If interspecific gene flow is limited, paraphyletic gene trees are neither unexpected nor problematic for species tree reconstruction methods (Schmidt-Lebuhn, 2011). However, this is not the case if hybridization is more pervasive. The concept of "species" used in species tree methods is one that fits uncomfortably with the suggested complex nature of many plant species, including *Ranunculus* (Hörandl & Emadzade, 2012). In such cases, there remains uncertainty over the interpretation of paraphyletic gene trees and whether or not discordant gene trees indicate complex relationships among species or incomplete lineage sorting characteristic of species radiations (Degnan & Rosenberg, 2009). Analytical approaches such as those outlined above provide new tools to better evaluate these trees as well as the extent and importance of hybridization in nature. If such study shows that hybridization is as significant as some authors have suggested, then there will be increased impetus to develop more appropriate coalescent models for species trees methodology (as already suggested: Brumfield et al., 2008). Alternatively, such a finding might stimulate the further development of approaches for reconstructing species relationships that are model free and based on criteria such as data partition concordance (e.g., see Larget et al., 2010) and/or those that get away entirely from Darwin's sticklike figure and consider heuristic solutions to the problem of reconstructing reticulate hybridization networks (e.g., Huson et al., 2005; as used in Pirie et al., 2009). Ultimately, the aim will be to better understand and describe the plant biodiversity in front of us, and progress will only come with improved understanding of the genetic data at hand. Developing this understanding will be an important contribution to the debate over whether or not to recognize paraphyletic species.

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NOTHING IN TAXONOMY MAKES SENSE EXCEPT IN THE LIGHT OF EVOLUTION: EXAMPLES FROM THE CLASSIFICATION OF *RANUNCULUS*[1]

Elvira Hörandl[2]

ABSTRACT

Biological classification aims at establishing ordering systems for organisms. Principles of classification, however, differ in their criteria and in their information content. Cladistic classification emphasizes information on descent, but the strict application of logical inclusiveness leads in practice to disregard of modification and to a lack of information on evolutionarily relevant features. Phenetics provides information on similarity regardless of descent. Evolutionary classification maximizes information on evolution by combining information on descent and modification, but it relaxes the requirement of inclusiveness. In practice, this means accepting holo- and paraphyletic taxa, but rejecting polyphyletic groups. Review of a recently published case study of the species-rich and cosmopolitan genus *Ranunculus* demonstrates how evolutionary classification can be performed in practice. A hypothesis of descent was reconstructed by phylogenetic analysis of DNA sequence markers plus morphological and karyological characters. Based on this backbone phylogeny, information content on morphology, karyology, and ecology was used as a criterion for delimitation of infrageneric taxa. This concept resulted in the subdivision of a more basal, paraphyletic *Ranunculus* subg. *Auricomus* and the holophyletic *Ranunculus* subg. *Ranunculus*. On a sectional level, 14 holophyletic and two paraphyletic sections plus one monotypic section were classified. Holophyletic sections mostly reflect extinction gaps, while paraphyletic groups appear in clades that have reticulate evolution and/or ecological shifts. Classification of paraphyletic and monotypic sections preserves information on morphology, ecology, and evolutionary processes. This pluralistic approach is justified as it best reflects the diversity of the genus. The principle of broadening criteria maximizes information on descent and modification. Evolutionary classification facilitates practicability and stability of taxonomic work, as the broadening of criteria restricts the number of equally valid options for classification. For users, preserving information content on phenotypes aids practicability, because the connection to traditional literature and to modern information systems is optimally maintained.

Key words: Classification, evolution, holophyly, paraphyly, phylogeny, practicability, Ranunculaceae, *Ranunculus*.

The title of this article on the classification of *Ranunculus* is inspired by Dobzhansky's (1973) famous statement that "Nothing in biology makes sense except in the light of evolution." This article discusses the importance of incorporating information on evolution into biological classification. Much has been disputed before on theory, and the issue of paraphyly has remained controversial (Ashlock, 1971; Duncan, 1980; Stuessy, 1987, 1997; Brummitt, 2002; Mayr & Bock, 2002; Ebach & Williams, 2004; Hörandl, 2007; Hörandl & Stuessy, 2010; Schmidt-Lebuhn, 2012, Stuessy & Hörandl, 2014). The current practice of classification, however, does not reflect the variety of opinions. Above the species level, most taxonomists adhere to principles of cladistic classification. In this article, I first briefly review the relationship of evolution, phylogeny, taxonomy, and their functions as information systems. The presentation of *Ranunculus* as a case study will hopefully reveal practicable solutions for evolutionary classification. Finally, I discuss some general points regarding contemporary taxonomy.

Evolution means "change," and therefore is a process. In the broadest sense, it is a process of populations undergoing descent with modification over generations. Contemporary taxonomy takes evolution partly into account as almost all modern authors agree that biological classification above the species level should reflect descent (e.g., Mishler, 2009; Hörandl & Stuessy, 2010). Descent is best reflected by the DNA molecules carrying heritable information from one generation to the next, and DNA sequence data have become the marker of choice for phylogenetic reconstruction of descent. Descent,

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however, also establishes patterns of similarity in descendants because of the shared inheritance of features. Modification, in contrast, is the raw material of variation and for selection to act upon, and therefore the base of change and diversification. Modification, however, exists at different levels of organismic organization—at the level of micro- or macromolecules, genes, chromosomes, enzymes, membranes, cells, organs, organisms, or behavior. These processes are not necessarily reflected in the currently used DNA sequence markers. In flowering plants, modification can have its background in gene expression patterns and epimutation, chromosomal change, gene duplication, or even whole genome duplication, and such modifications may have fundamental effects on phenotypes (e.g., Cubas et al., 1999; Soltis et al., 2009; Chanderbali et al., 2010). Moreover, population structure, community structure, and even ecological interaction do have effects on the phenotype via selection and even direct influence on epigenetic control mechanisms in complex feedback loops (e.g., Bossdorf et al., 2008). Therefore, modification cannot be so easily pinpointed to a certain type of data, and modification has often been neglected in phylogenetic systematics in theory and practice (see examples in other papers of this symposium). Modern cladistic classification regards only a minimum of information on modification, i.e., that of a synapomorphy, which is in practice restricted to DNA sequence data (the problem of a loss of information on character combinations has been discussed elsewhere; Hörandl, 2010).

Taxonomy aims at establishing ordering and information systems on organisms (Stuessy, 2009). In general, ordering systems are based on defined and well-circumscribed entities with information content, i.e., on classes. In biological classification, organisms are sorted into taxa according to certain criteria, with the aim that the taxon, as a class, has theoretical foundation, information content, and predictivity. The criteria for grouping determines the information content of a taxon (Fig. 1):

- Cladistics bears information on descent and accepts monophyletic (inclusive) groups only (better termed as holophyletic; see Hörandl & Stuessy, 2010, on terminology). In asymmetrically bifurcating or reticulate phylogenies, divergence via autapomorphies or reticulate evolution cannot be recognized (Hörandl, 2006). This concept bears the danger of loss of information on the modifications of the phenotype (Fig. 1B).
- Phenetics bears information on modification only, as modification (as a process) results in dissimilarity (as a pattern); inclusiveness according to the criterion of similarity is usually handled in a relaxed fashion according to practicability. Phylogenetic status is not relevant. Therefore, holo-, para-, and polyphyletic groups can be accepted. This concept suffers from lack of information on descent and from various competing options for grouping (Fig. 1C).
- Evolutionary classification bears information on descent plus modification and partly inclusiveness. It accepts holophyletic and paraphyletic groups, as paraphyletic groups do share ancestry, but are just not strictly inclusive. The concept also effectively reduces options for grouping (Fig. 1D).

For cladistic classification, information content on modification other than synapomorphies is simply not relevant. The information content of autapomorphy, symplesiomorphy, and conflicting characters is not being recognized. Instead, the focus on descent has driven taxonomists to a purely logical criterion, i.e., that of inclusiveness, which means that all descendants must be included. Inclusiveness is not necessarily a biological feature, but a construct of human thinking. From an evolutionary point of view, strict inclusiveness can never be achieved in biological systems, because this criterion would require a completely static system; evolution, however, is change, and change always means that transitions happen from one class to the next. Inclusiveness is needed for maximum predictivity of a group. However, it should be emphasized that most classes outside biology are not strictly inclusive, but do have fuzzy borders: colors, languages, species, community structures, religions, cultures—all these commonly used classes are not strictly inclusive, but nevertheless have predictivity (Holynski, 2011). No human language is logical and inclusive, but nevertheless works for carrying information. Also, for phenetics, inclusiveness has less priority, because similarity as the main criterion also often has fuzzy borders. Information content of a class, however, is not given by inclusiveness per se, but by the choice of criteria. To maximize information content on evolution, therefore, it is more important to broaden criteria (descent and modification) than to put emphasis on strict inclusiveness. Therefore, inclusiveness is not a primary criterion for evolutionary classification.

Evolutionary classification uses descent as a primary criterion to establish a backbone, and modification as a secondary one to make decisions for grouping (Fig. 1D). The priority is justified as shared ancestry usually causes similarities in the bauplan, simply because of shared inherited features, and this kind of similarity is usually informative about evolution. This method differs fundamentally

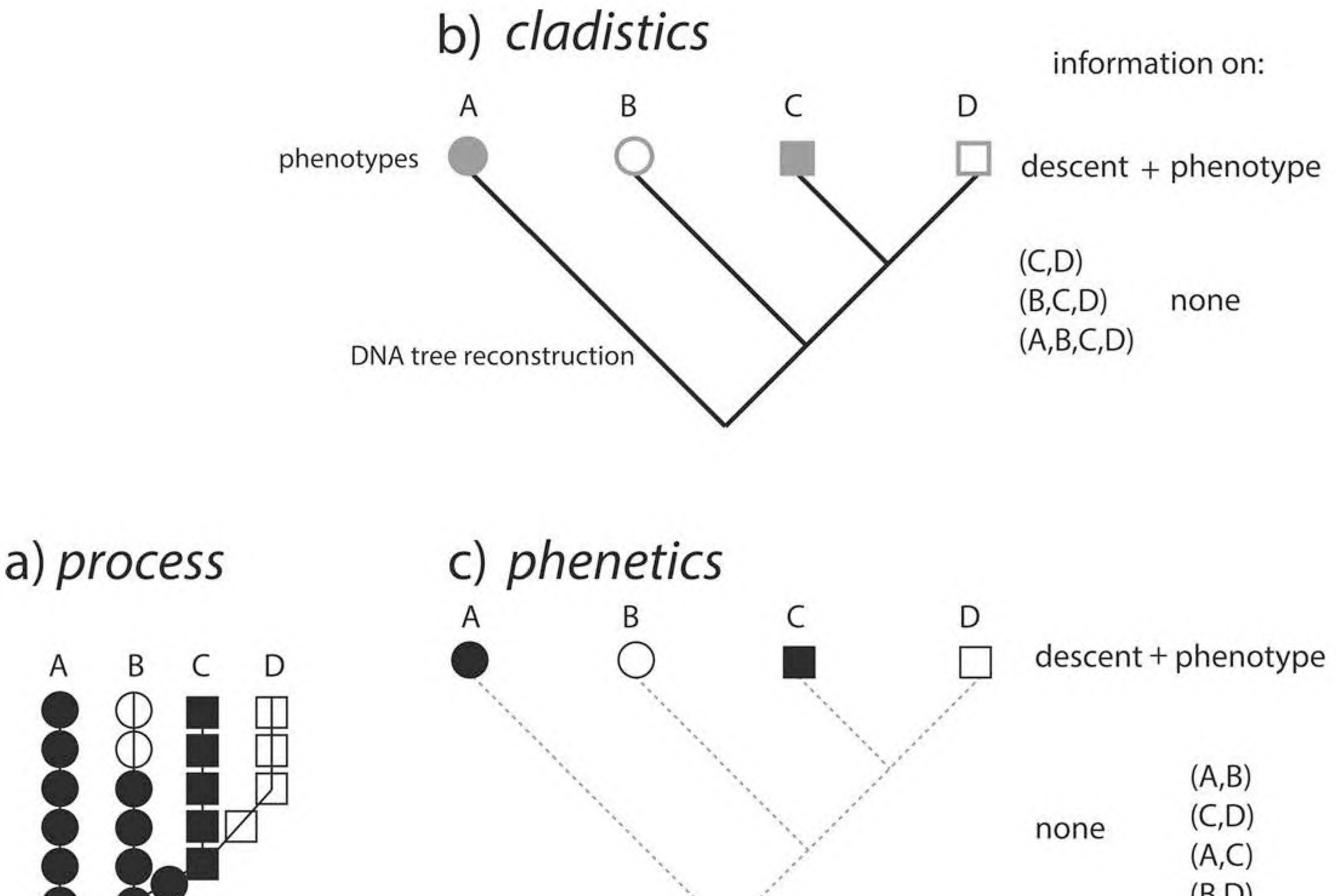


Figure 1. Scheme of information content of different classification concepts, with two characters each with two states mapped onto a phylogeny of four taxa (outgroup not shown). —A. Evolutionary process resulting in paraphyly (budding), with a phenotypic character evolving along lineages. B, C. Mapping of the character on a DNA sequence phylogeny and inferred grouping concepts. —B. Cladistics classification based on shared ancestry provides no information on phenotypic change and has three grouping options. —C. Phenetics based on the four symbols provides no information on descent and has four grouping options. —D. Evolutionary classification regards the phylogeny plus similarity and thus provides information on descent and modification; just two options for grouping are left.

from phenetics where similarity is the primary criterion, while descent was rather deduced from it in an interpretative, but not decisive fashion. Similarity as the primary criterion often has resulted in polyphyletic taxa in the past (for instance, plants with catkin-like inflorescences being classified as Amentiferae, which includes several, unrelated orders of angiosperms). Unfortunately, polyphyly and paraphyly have often been confused, and this confusion has been seen as a major argument against evolutionary classification. The problem arises from the inclusiveness principle of cladistics, as paraphyly and polyphyly apply to both non-inclusive groups, but otherwise rely on a fundamentally different *biological* criterion. A paraphyletic group is based on descent, it was once holophyletic, it once had a

shared common ancestor, and shared features (symplesiomorphies) are a result of this shared ancestry (i.e., a former synapomorphy); see Hörandl and Stuessy (2010). The paraphyletic group appears in tree topologies usually as a basal grade with one or more holophyletic derivatives descended from it. Paraphyletic and holophyletic taxa are thus "convex" in the sense of Duncan (1980), whereby a subset of evolutionary entities is convex "if the path of line segments between any two members of the subset does not include phyletic line segments that are part of a path connecting two evolutionary units of a different subset."

A polyphyletic group, in contrast, never had a holophyletic stage in its evolutionary history. It is not convex sensu Duncan (1980). Its shared features (homoplasies) are not a product of descent. Homoplasy might have different genetic backgrounds; in angiosperms, the overall capacity of rapid shifting between morphological character states is quite well documented, and often just due to changed expression patterns of the same genes (e.g., Chanderbali et al., 2010). Reconstructions of ancestral states using morphological data alone are complicated, but that can be done on DNA backbone trees (e.g., Endress & Doyle, 2009). The members of a polyphyletic group would appear separated by several nodes in a molecular tree topology. (Perhaps it might be better to apply the term "aphyletic" to such groups as, in fact, there is no genealogy in terms of descent involved.) The term "polyphyletic" may be better applied to cases of multiple hybrid origins of a taxon, or segregation of characters in hybrid progenies; such cases also may produce a scattered distribution of character states onto a tree, but these patterns do have a genealogical background.

Classification of the genus *Ranunculus* exemplifies how evolutionary classification can maximize the information content of taxa. The genus is highly diverse and widespread, comprising about 600 herbaceous species (plus ca. 600 agamospecies), with a cosmopolitan distribution (Tamura, 1995; Hörandl et al., 2005; Emadzade et al., 2010). Variation and diversity exist at many different organizational levels. The present state of research has been summarized by Hörandl and Emadzade (2012): DNA sequence data (*matk/trnk*, *psbJ–petA*, and nrITS) are available for ca. 300 species, i.e., about half the genus. Chromosome counts and ploidy information are available for about one third of the species, while the great variation of the karyotype within the genus has been studied in detail for about 100 species (Goepfert, 1974; D'Ovidio & Marchi, 1990; Dahlgren, 1991; Baltisberger, 1994; Takahashi, 2003; Baltisberger & Widmer, 2009). Polyploidy occurs in ca. 40% of species and plays a major role in the diversification of the genus (Hörandl et al., 2005; Hoffmann et al., 2010; Hörandl & Emadzade, 2012). The morphological phenotype has been described for ca. 600 species; morphological data compiled with a stringent and comparable methodology for phylogenetic analysis have been compiled for ca. 200 species (Hörandl & Emadzade, 2012). Geographical distribution and related ecology are more or less known for all described species (summaries in Tamura, 1995; Hörandl & Emadzade, 2012). Ecological diversity is in general extraordinarily high, with species occurring both in freshwater and terrestrial habitats, ranging from semi-deserts to temperate forests and grassland, arctic-alpine tundra, from montane rainforests to anthropogenic habitats (Tamura, 1995). Because of its cosmopolitan distribution, the genus is represented in most regional and local bioinformation systems (floras, databases, and checklists) of the world.

The evolutionary history of the genus is fairly diverse. Recent biogeographical studies have demonstrated that long-distance dispersal and multiple colonizations of continents have shaped the evolutionary history of the genus (Emadzade & Hörandl, 2011; Emadzade et al., 2011). *Ranunculus* originated about 21 million years ago (Ma) and has a crown group age of ca. 18 million years (My); the genus diversified later on during the Cenozoic in several waves via various evolutionary processes. Many high mountain clades originated and diversified during the Quaternary (Emadzade & Hörandl, 2011) under the influence of Pleistocene glaciations. Hybridization occurs in many extant groups with or without polyploidy (Cook, 1966; Huber, 1988; Lockhart et al., 2001; Hörandl et al., 2005, 2009). Reticulate evolution may have also happened frequently in the evolutionary history of the genus, as it can be hypothesized from incongruent placements of some major subclades (e.g., *Ranunculus* sect. *Thora*) within the phylogeny in nuclear and plastid datasets (e.g., Paun et al., 2005).

Classification of *Ranunculus* should provide information on various evolutionary processes and organismic levels that have shaped the diversity of the genus. Neither morphology alone, nor a DNA-based phylogeny alone, could provide this information. Previous similarity-based infrageneric classifications of *Ranunculus* were always controversial, and traditional sections by Tamura (1995) appeared to be scattered over molecular trees (Hörandl et al., 2005). The infrageneric taxa show a mosaic-like pattern of character combinations, mostly without unique

features, which has led to various taxonomic concepts, depending on the emphasis on selected characters (Hörandl & Emadzade, 2012). On the other hand, using information only on descent would make a classification of *Ranunculus* quite meaningless, as evolution acts in various ways, and modification plays a major role in diversification. Information is needed on karyology, phenotype, character evolution, and ecology to reflect the diversity of the genus. An infrageneric classification of subgenera and sections tries to meet these demands (Hörandl & Emadzade, 2012). Here I summarize the evolutionary history and features of accepted sections (Table 1) and review the new classification also with respect to different historical, theoretical, and practical aspects. A main goal of my discussion is to demonstrate the superiority of a pluralistic approach for classification over a strictly dogmatic theoretical principle. From this aspect, paraphyletic taxa are, in terms of information content, acceptable for classification if they bear information on structure, function, evolution, and ecology (see Hörandl & Stuessy, 2010).

Evolutionary History and Infrageneric Taxa of *Ranunculus*

Ranunculus s. str. (sensu Emadzade et al., 2010) has been confirmed as holophyletic in all previous phylogenetic studies based on DNA data (Hoot, 1994; Johansson, 1995, 1998; Ro et al., 1997; Hörandl et al., 2005; Paun et al., 2005; Lehnebach et al., 2007; Gehrke & Linder, 2009; Emadzade et al., 2010; Hoffmann et al., 2010). The circumscription of the genus has recently been settled based on molecular and morphological data (Emadzade et al., 2010). This classification avoided a polyphyletic genus *Ranunculus* as it has been revealed by the molecular phylogenetic study by Hoot et al. (2008), which had focused on the position of *Hamadryas*. The only solution of a holophyletic genus *Ranunculus* sensu Hoot et al. (2008) would have been to merge all genera of the tribe into one genus. Such an extreme lumping would not reflect the diversity of the tribe and is not accepted by Eurasian and South American authors who emphasize morphological diversity on the generic level (e.g., Lehnebach et al., 2007; Wang et al., 2009). The classification by Emadzade et al. (2010) is to date the only solution of a holophyletic genus *Ranunculus*. Previous classifications based on morphology only rendered *Ranunculus* as polyphyletic (e.g., Tamura, 1995; Whittemore, 1997). The infrageneric classification by Hörandl and Emadzade (2012) was based on a phylogenetic backbone and a minimum of shared morphological characters of each group. We subdivided the genus into a paraphyletic *Ranunculus* subg. *Auricomus* and a monophyletic *Ranunculus* subg. *Ranunculus*, and we discriminated 17 sections, 14 of them being holophyletic, two paraphyletic, and one monotypic; we did not accept any of the previous polyphyletic sections. The acceptance of paraphyletic groups and one monotypic section was mostly justified by morphological and ecological information content.

Holophyletic Sections: Extinction Gaps

Holophyly is a stage of evolution when the paraphyletic stem group, from which the ancestor arose, has gone extinct. Holophyletic groups, therefore, reflect historical extinction gaps (Hörandl & Stuessy, 2010). Most holophyletic groups can be related to biogeographical scenarios with massive extinction due to climatic changes. Nine of the 14 clades classified as sections occur predominantly in high mountain systems of the temperate to arctic zones and comprise mostly high alpine species (sections *Thora*, *Aconitifolii*, *Ranuncella*, *Epirotes*, *Leucoranunculus*, *Auricomus*, *Pseudadonis*, *Oreophili*, *Euromontani*; see Hörandl & Emadzade, 2011). Our dating analysis suggests that the origin of all these clades predates the Pleistocene (Emadzade & Hörandl, 2011). Glaciations and climatic cooling during the Pleistocene likely contributed to the extinction of paraphyletic stem groups, while later diversification and speciation happened mostly among cold-adapted taxa of alpine zones. Sections *Aconitifolii* and *Ranuncella* also include species from lower altitudes of the same geographical region (Paun et al., 2005). A global comparison of alpine taxa revealed that species from different mountain systems are related to species of adjacent lowlands rather than to species of mountain systems in other areas, which suggests that alpine groups have arisen independently several times from lowland ancestors (Hörandl & Emadzade, 2011). In previously glaciated areas, some holophyletic groups comprise only alpine species (sections *Epirotes*, *Leucoranunculus*, *Pseudadonis*); their lowland ancestors might have gone extinct (Paun et al., 2005; Hörandl & Emadzade, 2011). The shift to higher altitudes and ecological adaptation to alpine conditions may have established reproductive isolation among the ancestors of these clades in early stages of their evolutionary history, while geographical isolation and vicariant speciation were main drivers of speciation within clades.

Ranunculus sect. *Flammula* represents a holophyletic group with a strong ecological preference for wetlands and water habitats at lower altitudes. As all clade members share this ecology, it can be

Table 1. Survey of the accepted sections of *Ranunculus* (after Hörandl & Emadzade, 2012) and hypotheses on their evolution.

Nr.	Name	Phylogenetic status	Evolutionary origin	Main internal diversification processes	Key references
1	sect. *Thora*	holophyletic	ancient hybrid origin, Pleistocene extinction gap of stem group	allopatry	1, 2, 3, 4
2	sect. *Aconitifolii*	holophyletic	uncertain	adaptive radiation, also polyploidy	1, 2, 3, 4, 5
3	sect. *Ranuncella*	holophyletic	geographical and ecological isolation (orophytic)	radiation on Iberian Peninsula	1, 2, 3, 4, 5
4	sect. *Epirotes*	holophyletic	geographical isolation (orophytic)	ongoing speciation/gene flow	1, 2, 3, 4, 5
5	sect. *Leucoranunculus*	holophyletic	geographical and ecological isolation (orophytic)	allopatry, vicariant speciation	1, 2, 3, 4, 5
6	sect. *Pseudadonis*	holophyletic	early geographical isolation on New Zealand (orophytic)	hybridization, polyploidy, adaptive radiation	1, 2, 3, 6
7	sect. *Batrachium*	holophyletic	hybrid origin out of wetland group	ongoing hybridization, polyploidy, adaptive radiation	1, 2, 3, 7
8	sect. *Hecatonia*	paraphyletic	widespread stem group of former section	geographical isolation	1, 2, 3
9	sect. *Auricomus*	holophyletic	ancient extinction gap	hybridization, polyploidy, apomixis	1, 2, 3, 8
10	sect. *Flammula*	holophyletic	ancient extinction gap	long-distance dispersal, peripatry	1, 2, 3
11	sect. *Ranunculus*	holophyletic	dysploidy event	long-distance dispersal, geographical isolation, polyploidy	1, 2, 3, 9, 11
12	sect. *Polyanthemos*	holophyletic	ancient extinction gap	long-distance dispersal, geographical isolation, polyploidy	1, 2, 3, 11
13	sect. *Echinella*	monotypic	anagenetic, character evolution	—	1, 2, 10, 11
14	sect. *Trisecti*	holophyletic	uncertain; geographical isolation	no information	1, 2, 11
15	sect. *Oreophili*	holophyletic	geographical isolation (orophytic)	geographical isolation	1, 2, 3, 10, 11
16	sect. *Euromontani*	holophyletic	geographical and ecological isolation (orophytic)	adaptive radiation, also polyploidy	1, 2, 3, 10, 11
17	sect. *Ranunculastrum*	paraphyletic	Tethyan stem group of 16; geographical and ecological isolation (partly orophytic)	geographical isolation, ecological shifts	1, 2, 3, 10, 11

[1] Hörandl and Emadzade (2012).
[2] Emadzade et al. (2010).
[3] Hörandl et al. (2005).
[4] Hörandl and Emadzade (2011).
[5] Huber (1988).
[6] Lockhart et al. (2001).
[7] Cook (1966).
[8] Hörandl et al. (2009).
[9] Goepfert (1974).
[10] Paun et al. (2005).
[11] Emadzade et al. (2011).

hypothesized that the most recent common ancestor was a hydrophilous plant. The strong ecological specialization may have eliminated a less specialized and less competitive paraphyletic stem group. This clade combines conserved morphological features from *Ranunculus* subg. *Auricomus* (swollen achenes) and *Ranunculus* subg. *Ranunculus* (foveolate microstructure of achene surface); see Hörandl and Emadzade (2012, supplement 5). Whether this combination is adaptive or due to reticulate evolution requires further study.

Chromosomal change is another putative explanation for holophyly. In section *Ranunculus*, the origin of the clade can be related to karyotype evolution and a dysploidy event with a shift to a chromosome base number from $x = 8$ to $x = 7$. *Ranunculus* sect. *Polyanthemos*, a highly diverse and widespread clade of lowland to mid-altitude species, is sitting on a relative short branch and is also morphologically not strongly differentiated from its sister clade (Hörandl & Emadzade, 2012). *Ranunculus* sect. *Trisecti* from the Caucasus-Irano Turanian region is not well known; further studies are needed to understand its evolutionary history.

Paraphyletic Sections and Their Holophyletic Derivatives

Two paraphyletic ecological groups were accepted by Hörandl and Emadzade (2012): *Ranunculus* sect. *Hecatonia* and *Ranunculus* sect. *Ranunculastrum*. They have different evolutionary histories. In both cases Hörandl and Emadzade (2012) could demonstrate that information content of paraphyletic taxa on morphology, ecology, and evolutionary process exceeds that of alternative cladistic classifications.

Ranunculus sect. *Hecatonia* forms a grade at the base of *Ranunculus* sect. *Batrachium* (Fig. 2); the relationship of some species to New Zealand alpine species (*Ranunculus* sect. *Pseudadonis*) may point at an older biogeographical connection via Antarctica. In all three sections, polyploidy is frequent and reticulate evolution has been documented (Fig. 3). However, so far we cannot see a clear progenitor-derivative relationship of certain diploid and polyploid taxa, but rather a wide-meshed network with one well-supported genetic cluster, *Ranunculus* sect. *Batrachium*, emerging out of it (see above). Polyploidy and multiple copies of the ITS of the nuclear ribosomal DNA are probably responsible for numerous conflicts between gene trees from different loci (Hörandl et al., 2005). *Ranunculus* sect. *Batrachium* is holophyletic in the total evidence approach, but not according to molecular data alone (Emadzade et al., 2011). Reticulate evolution, especially allopolyploidy, is probably the reason for equivocal results on shared ancestry. Neighbor-net analysis based on genetic distances (molecular combined dataset from Hörandl & Emadzade [2012]) indicates that several splits contribute to phylogenetic incompatibilities (see scale bar in Fig. 3). However, clustering of species of *Ranunculus* sect. *Batrachium* (Fig. 3) shows the genetic similarity within *Ranunculus* sect. *Batrachium*. The group might well have originated from an ancient hybridization event, but recent hybridization with members of other related sections has never been reported. The constancy of shared features (white flowers without a starch layer, U-shaped to ringlike to pyriform nectaries, wrinkles on the achene surface) speaks against a hypothesis of segregation of patterns in a hybrid swarm, but rather suggests a shared acquisition of these features together with the specialization to the aquatic habitat. Other characters, like creeping or floating habit, heterophylly, and the formation of dissected leaves in some taxa, are likely adaptive to the aquatic habitat, and show plasticity according to environmental conditions (Cook, 1966).

The problem is that recognizing *Ranunculus* sect. *Batrachium* leaves a paraphyletic group, *Ranunculus* sect. *Hecatonia*. Species of *Ranunculus* sect. *Hecatonia* differ from *Ranunculus* sect. *Batrachium* by mostly yellow petals with a starch layer and a smooth achene surface (except for *R. sceleratus*, with sometimes weakly wrinkled achenes). They colonize a broad range of habitats from wet soil in bogs and along ponds to semiaquatic habitats; only a few species are found in freshwater habitats like *Ranunculus* sect. *Batrachium* and do exhibit the typical habit of water-buttercups (floating, procumbent, and heterophyllous with dissected leaves). *Ranunculus* sect. *Pseudadonis* is holophyletic in Paun et al. (2005), in Hoffmann et al. (2010), and in the total evidence approach by Hörandl and Emadzade (2012). It comprises alpine terrestrial species of the high mountains of New Zealand and is morphologically distinct from the two former sections, *Ranunculus* sect. *Batrachium* and *Ranunculus* sect. *Hecatonia*, by erect habit, basal rosette leaves, and the long beaks of the achenes (Fisher, 1965). The whole group is hexaploid and relationships among species within the section are highly reticulate (Lockhart et al., 2001). Hybridization and adaptive radiation in various ecological niches in the New Zealand mountain systems have probably shaped the diversity of the group.

The acceptance of a paraphyletic section *Hecatonia* preserves information on the complex evolutionary history, on adaptive character evolution, and on

the ecology of three different classes: each section can be characterized by six to seven shared characters, and by a distinct ecology and evolutionary history (see Fig. 2; details in Hörandl & Emadzade, 2012). We regard this approach as more informative than the classification of a single clade (III), which would have no shared morphological characters, no predictive quality, and no other information content other than shared ancestry.

The evolutionary history of the paraphyletic *Ranunculus* sect. *Ranunculastrum* has been shaped by the biogeographical history of the ancient Tethyan area (Fig. 4). Phylogenetic and biogeographical analysis by Emadzade et al. (2011) suggests that the orophytic *Ranunculus* sect. *Oreophili* is probably the most ancient group. *Ranunculus* sect. *Ranunculastrum* has already been recognized by most previous authors as a distinct taxon, variously treated as a section, subgenus, or genus (Tamura, 1995, and literature therein), characterized by tuberous roots and elongated receptacles. Most species are found in the Mediterranean and Irano-Turanian regions, with single species occurring in Macaronesia or the Himalayas (Emadzade et al., 2011). Within this clade, the rhizomatous, tuber-lacking *Ranunculus* sect. *Euromontani* with mostly globose fruiting heads (collective fruits) emerged in the European mountain system, thereby rendering *Ranunculus* sect. *Ranunculastrum* paraphyletic (Fig. 5).

A Neighbor-net analysis of molecular data taken from Emadzade et al. (2011) of these two sections and their closest outgroup, *Ranunculus* sect. *Oreophili* (see Fig. 2), was conducted to gain insight into the evolutionary histories of these species (Fig. 5). The analysis shows a narrowly meshed network, without any bootstrap support for alternative splits. In contrast to clade III, a hypothesis of reticulate relationships is not supported, and related species are mostly allopatric or parapatric. Several genetic clusters corresponding to geographical groups appear from this ancestral backbone, but these clusters are only partly congruent with morphological characters connected to two major ecological shifts: the separation of *Ranunculus* sect. *Oreophili*/*Ranunculus* sect. *Ranunculastrum* probably reflects an ancient geographical bifurcation, while the emergence of the orophytic, summer-wet adapted *Ranunculus* sect. *Euromontani* made the group of species of the lowland to mid-altitude, summer-dry adapted *Ranunculus* sect. *Ranunculastrum* paraphyletic. Again, merging of these three groups into one or two clades would just result in a loss of information on biogeographical history, morphology, and ecology (Figs. 2, 4).

Polyphyly

Ranunculus also provides good examples for the discrimination of polyphyly and paraphyly. Most of the traditional morphology-based sections recognized by Tamura (1995) turned out to be polyphyletic, or perhaps better, aphyletic, in our phylogenetic analysis (Hörandl et al., 2005; Paun et al., 2005; Hörandl & Emadzade, 2012). The lack of unique synapomorphies for sections, and the mosaic-like character combinations for clades, very much correspond to the scheme shown in Figure 1C. Clades that seem similar based on one character will seem distant based on others, and there are no strong overall patterns of similarity when all characters are applied together. For this reason, although different authors have recognized many of the same subgroups in *Ranunculus*, they have often postulated significantly different relationships among them, resulting in significantly different delineations of the genera in Ranunculeae. This result demonstrates the weakness of purely phenetic approaches for classification in groups that lack unique synapomorphies for the main internal clades. The most obvious example might be the former section *Echinella*, which united several unrelated annual taxa with spines or bristles on the achenes. Presence of spines, bristles, or papillae is highly homoplasious within the genus and can vary even within the same species, but appears mostly in species of dry areas. Life form in *Ranunculus* is variable even within species (Cook, 1966), and the switch to an annual life form happened probably multiple times independently in summer-dry regions with short rainy seasons where a short life cycle is advantageous (Paun et al., 2005; Emadzade et al., 2011).

Monotypic Taxa

Recognizing a single species as a taxon poses the logical problem that a single entity (species) is not a "set," and the same entity must be classified at different levels of the hierarchy (Gregg's paradox; e.g., Buck & Hull, 1966). Many proponents of cladistic classification have regarded monotypic taxa as unacceptable. Evolutionary classification, however, can take a more relaxed approach to the problem as the criterion is not inclusiveness but the amount of modification. Retrospectively, we can trace this change as degree of divergence from the sister taxon. Through their phylograms, Emadzade et al. (2010, 2011) show that genera related to *Ranunculus* display a high degree of divergence, and recognize nine monotypic genera within Ranunculeae (2010). In the phylogram of the total evidence approach in Hörandl

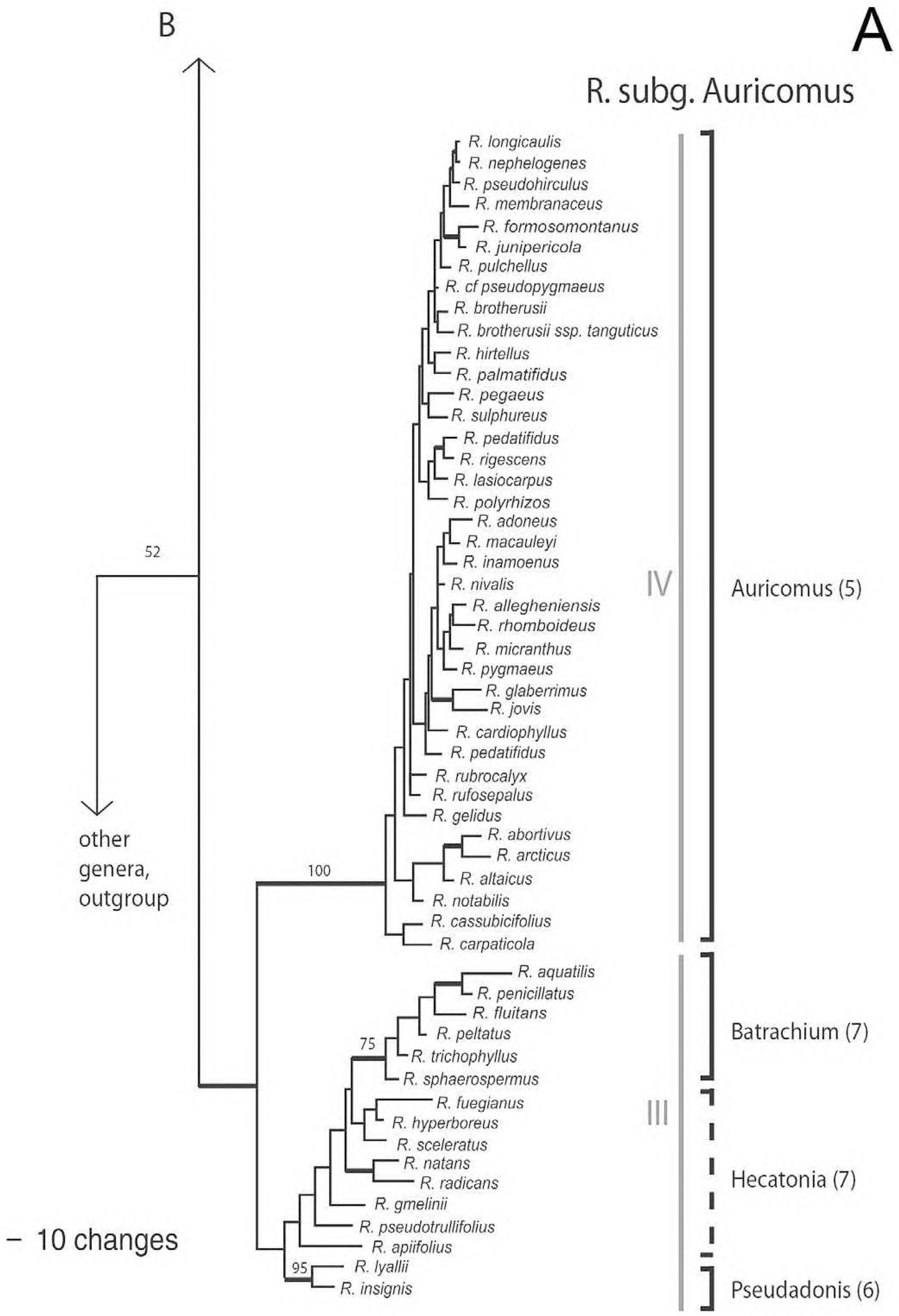


Figure 2. Phylogram of the total evidence analysis (MP) of the combined molecular (sequences of ITS of nrDNA, *matk*, *trnk*, and psbj-petA), morphological (44 characters) and karyological (2 characters) datasets based on 226 samples (outgroup taxa and other genera not shown; tree reprinted from Hörandl & Emadzade, 2012, with modifications). Branches with bootstrap values over 50% are in boldface (for clades representing a section, the bootstrap values are given above the branches). —A. Basal part of the tree belonging to paraphyletic *Ranunculus* subg. *Auricomus*. —B. Upper part of the clade representing *Ranunculus* subg.

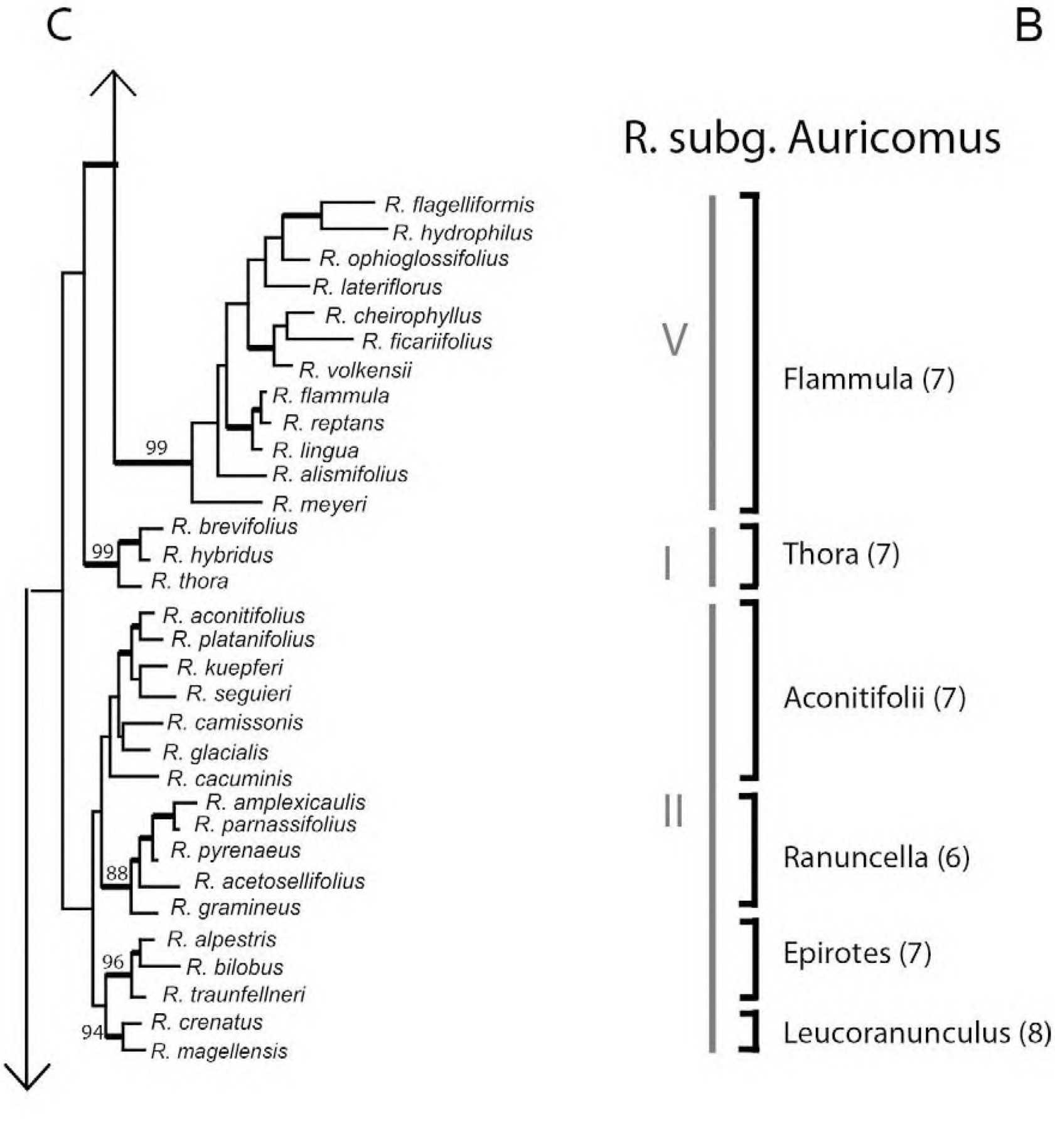


Figure 2. Continued.

and Emadzade (2012), it is clearly evident from branch lengths that divergence among genera is much higher than that among species of *Ranunculus* (Fig. 2). Alternative phylogenetic analyses based on a less complete sampling of genera of the tribe have rendered *Ranunculus* as polyphyletic (Hoot et al., 2008); the authors discussed the possibility of merging all genera of the tribe but did not draw any final formal conclusion. Such a lumping concept does not provide solutions as any taxon is nested in another and emerges out of it; finally *Ranunculus* might end up in a genus including all angiosperms (the "bony fish syndrome"; Brummitt, 2006). Cladistic classification is solely based on branching patterns and does not provide any information on divergence inside clades. Evolutionary classification, in contrast, regards synapomorphies and autapomorphies as crucial criteria for the delimitation of genera (see Stuessy et al., 2014). Interestingly, a high degree of morphological and genetic divergence occurs among genera of Ranunculeae in combination with long-distance dispersal (Emadzade & Hörandl, 2011), which also applies to endemic genera of oceanic islands (see Stuessy et al., 2013). Within *Ranunculus* s. str., such divergence patterns of island endemics are uncommon, at least for morphological divergence. Sections are usually not characterized by acquisition of unique morphological characters along

←

Auricomus. —C. Lower part of the clade representing *Ranunculus* subg. *Ranunculus*. —D. Upper part of the clade representing *Ranunculus* subg. *Ranunculus*. Dashed brackets, paraphyletic sections; solid brackets, holophyletic sections, with names of sections indicated on the right. The numbers in brackets next to section names indicate the number of shared morphological and karyological characters for that section. Gray bars with Roman numerals refer to clade numbers of the molecular tree only (see details in Hörandl & Emadzade, 2012). Scale bar indicates number of changes.

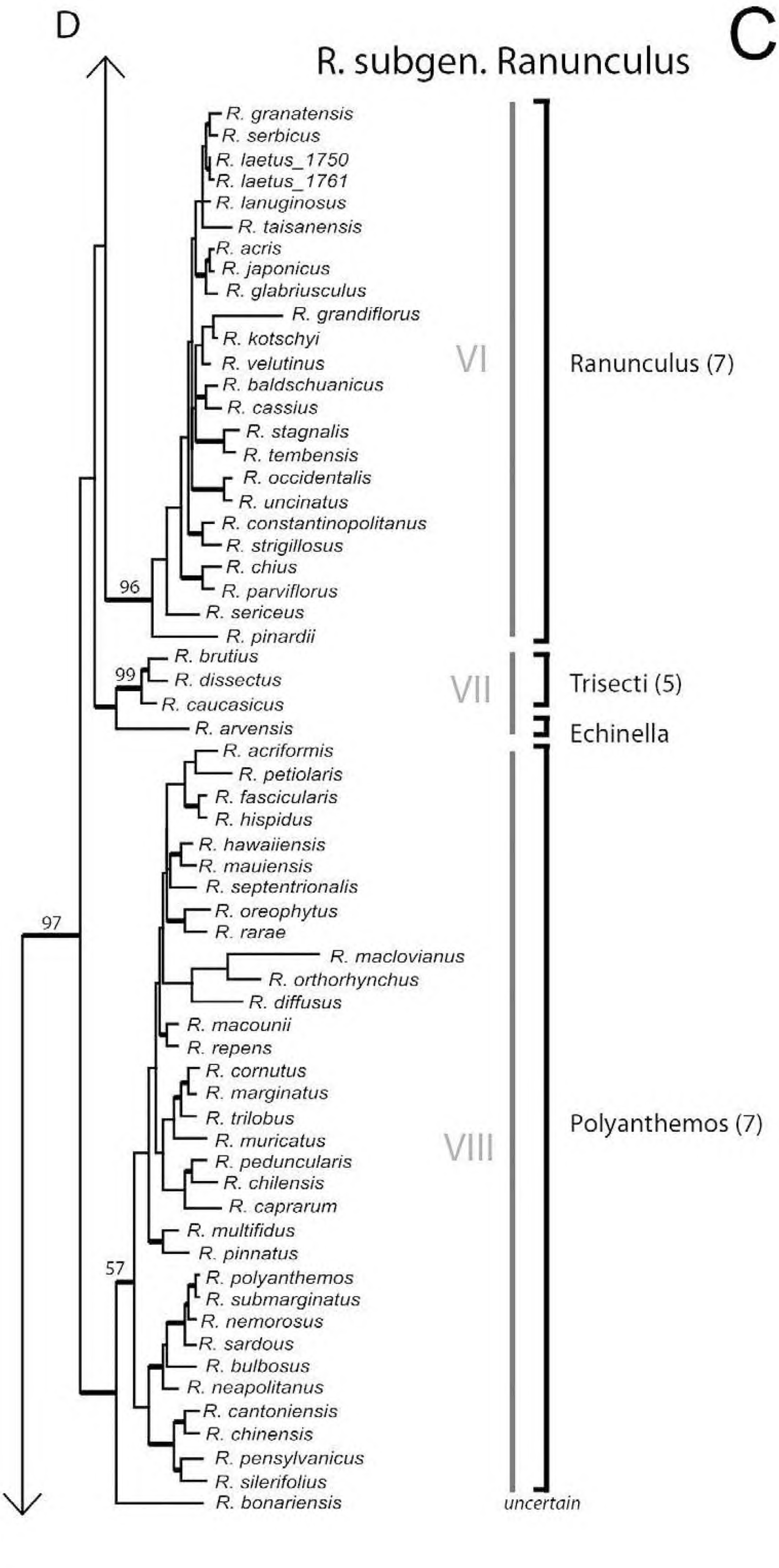


Figure 2. Continued.

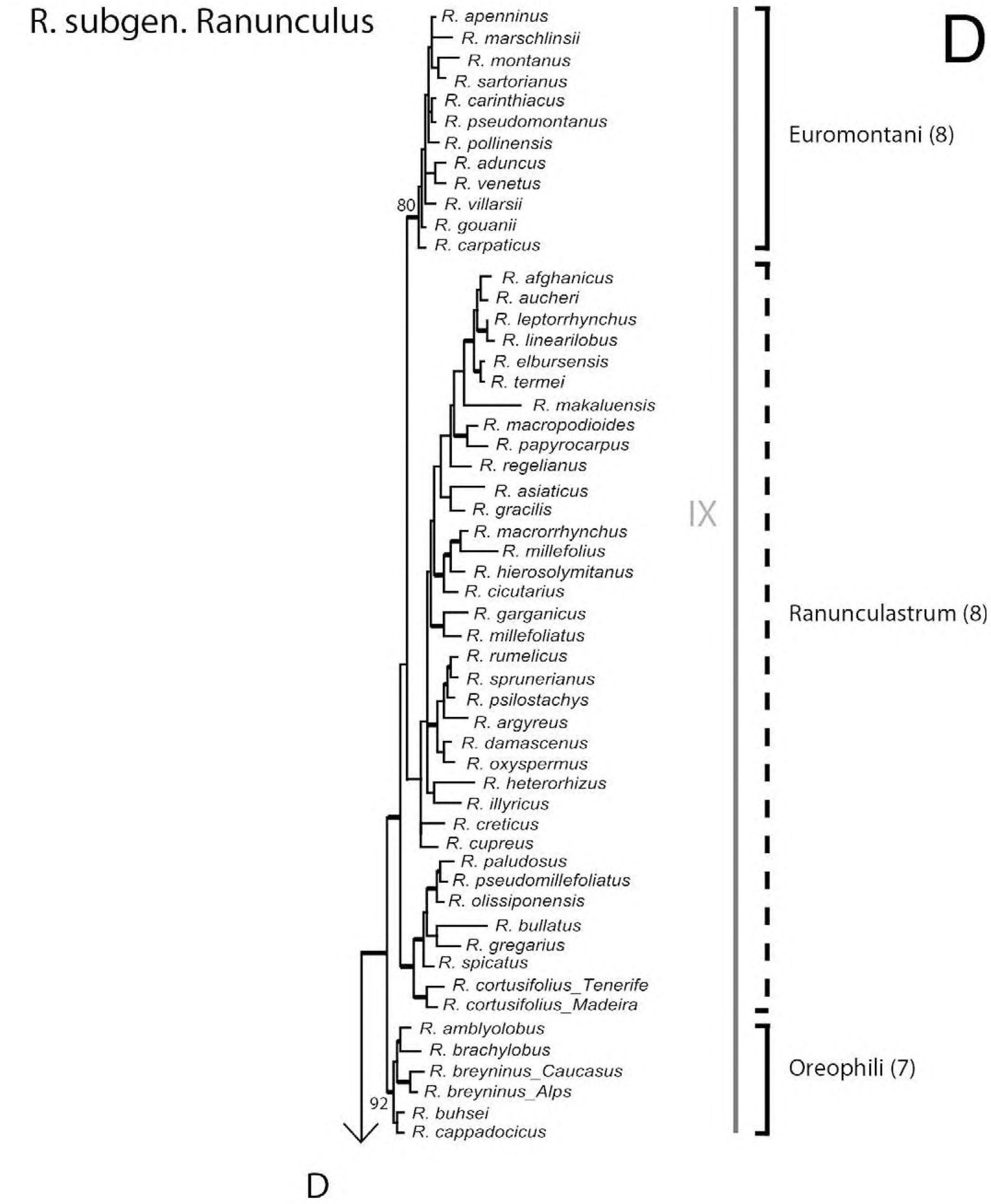


Figure 2. Continued.

a branch; instead, they seem to move between a few common character states, producing a mosaic-like pattern of similarities (Hörandl & Emadzade, 2012).

Ranunculus arvensis is a species with a unique combination of character states: it is an annual with very large, flat achene bodies that are covered with long, sharp spines, a fine papillose microstructure of achene surfaces (Emadzade et al., 2010), and large pollen grains with tricolpate to pantoporate aperture types (Santisuk, 1979). The species is placed on a basal branch of a morphologically heterogeneous clade of species from the Caucasus area and the Irano-Turanian region (VII). Interestingly, *R. arvensis* has a unique, tetraploid karyotype within the genus, consisting of 32 chromosomes of extremely unequal size, and composed of four haploid sets of 4-2-2 or 5-

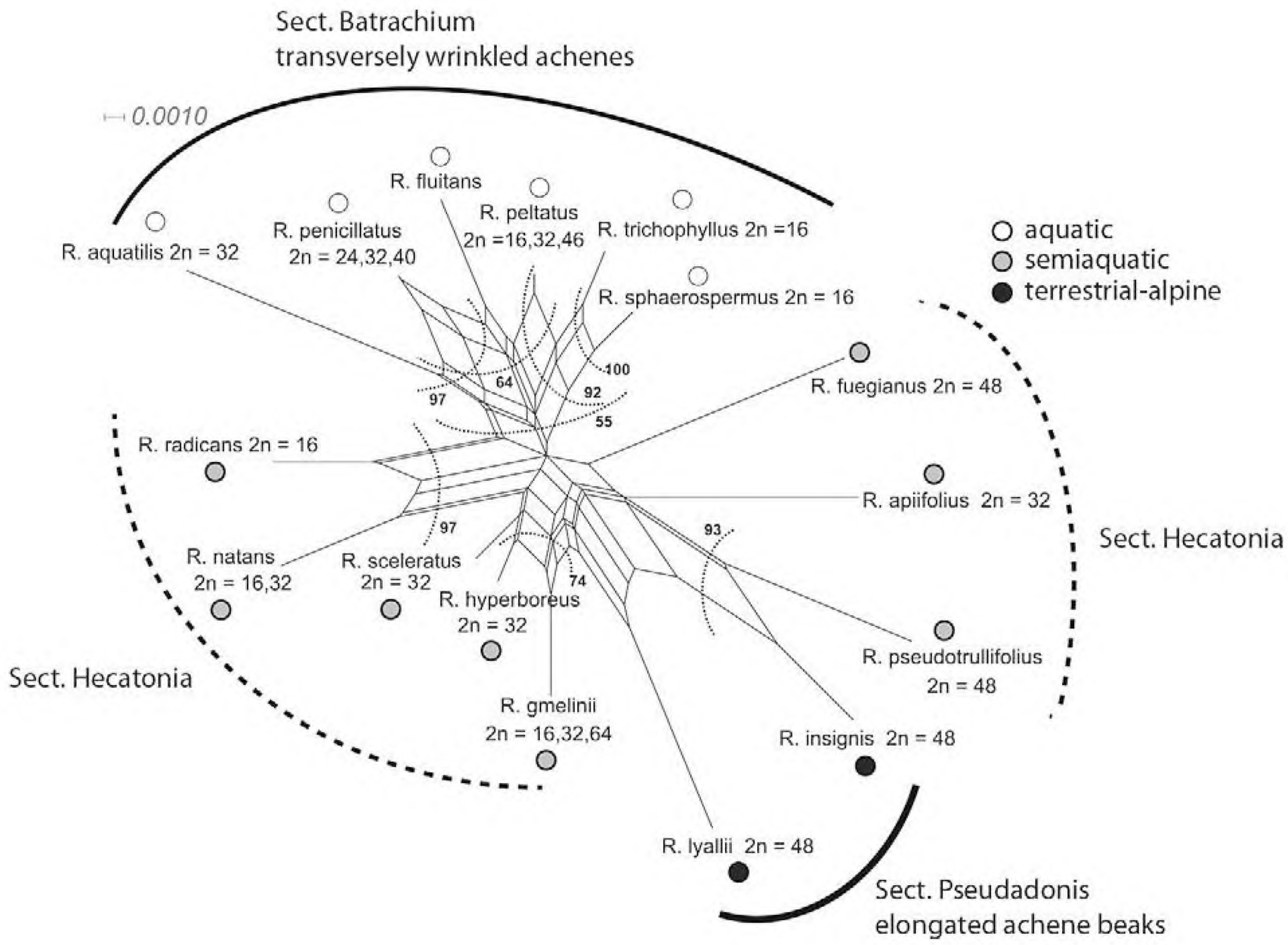


Figure 3. Neighbor-net analysis of clade III based on the molecular dataset only (see Fig. 2; redrawn from Hörandl & Emadzade, 2012), with habitat and karyological information added. Bootstrap values > 50% are shown for main clusters. Scale bar indicates number of changes.

1-2 (acrocentric-submetacentric-metacentric) chromosomes, respectively. The species is probably an allotetraploid of rather unique derivation (Goepfert, 1974). Unfortunately, cytogenetic data are not available for its closest relatives (*Ranunculus* sect. *Trisecti*) and are also not complete enough for phylogenetic analysis. However, a unique karyotype has also been a major criterion for recognizing monotypic taxa in other genera (Stuessy, 2009: 167). Altogether the classification of *R. arvensis* must be regarded as provisional as the information on its sister group is limited, but the present evidence points at a quite isolated position of *R. arvensis* within *Ranunculus*. Our treatment confirms the view of Davis (1960) who proposed that *R. arvensis* should be best treated as a monotypic section.

The information content of a monotypic taxon is mostly that of pronounced divergence (see also Stuessy & König, 2008; Stuessy et al., 2013). It is less relevant whether this divergence is due to an extinction of sister species, leading to a monotypic genus that is relictual, or whether the taxon itself has undergone rapid divergence or even saltational speciation, which may happen in allopolyploids. In both cases, monotypic taxa bear information on evolutionary change, which justifies this type of classification despite some logical inconsistencies.

Discussion

Our classification of *Ranunculus* follows a pluralistic principle: we weigh shared ancestry, genetic and phenotypic similarity, divergence, as well as ecological features, on a case-by-case basis. The varying emphasis on different biological criteria and the lower priority of purely logical criteria (inclusiveness) may be a major point of criticism. However, inclusiveness is meaningless if not connected to biological information.

What kind of information is wanted? Information on descent and shared ancestry is certainly meaningful for understanding evolution, but it is necessarily hypothetical. Moreover, information on descent is in most cases based on a single level of organismic organization, i.e., that of DNA sequences, only; the epigenetic, karyological, and genomic levels are usually not regarded (oddly enough, most authors do not deal with ploidy level variation, although this includes whole genome duplications with all its consequences!). Hence, the biological information content of a clade postulated from sequences of one or two loci remains low, and in tree topologies offers various options for classifying different clades. Clades alone, as groups of descendants, are not yet biological classes, and "cladification" is not yet a classification (Mayr & Bock, 2002). Accepting paraphyletic taxa

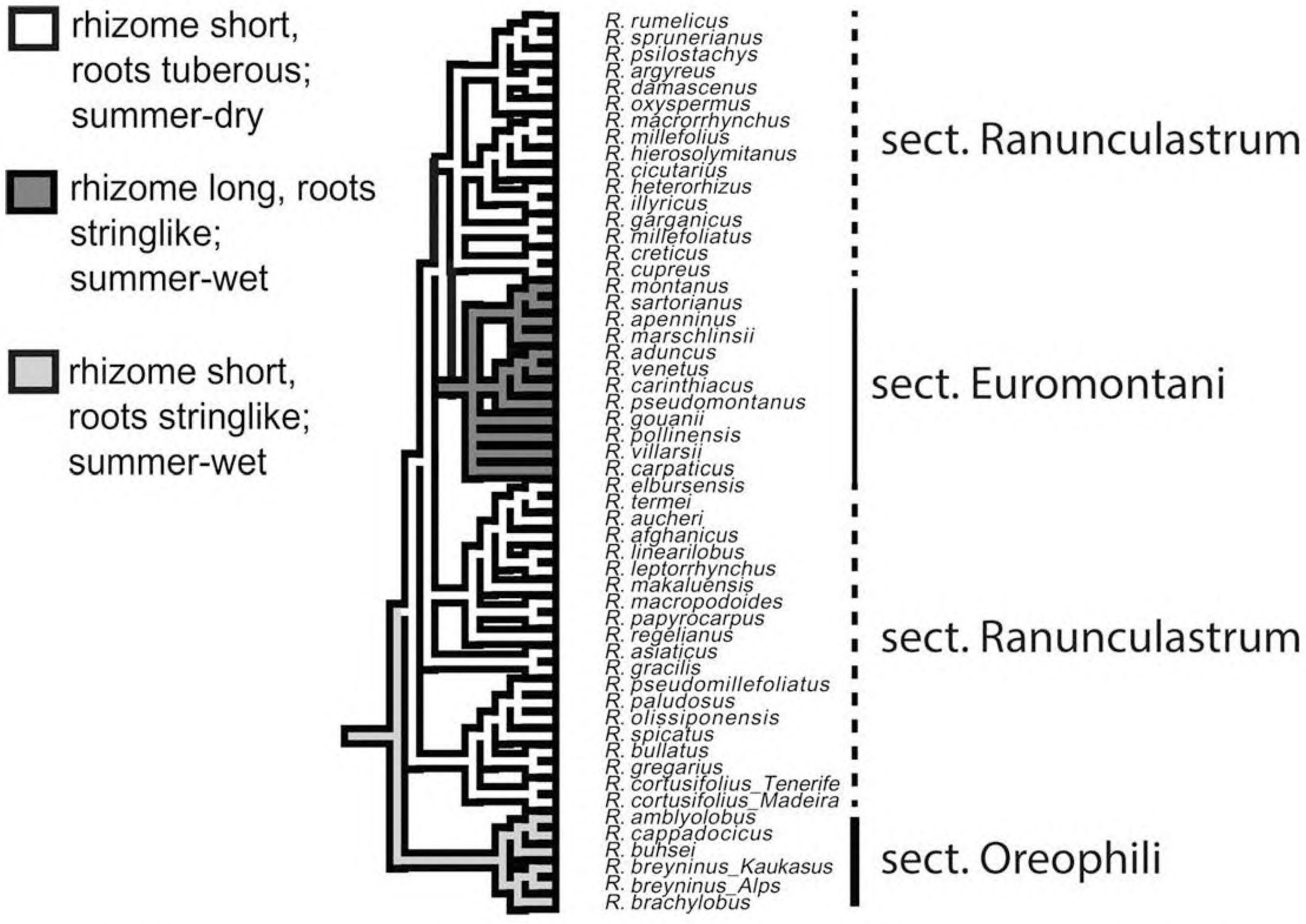


Figure 4. MacClade reconstruction (Maddison & Maddison, 2000) of underground parts mapped onto the molecular tree, clade IX (see Fig. 2D; all data from Hörandl & Emadzade, 2012).

means increasing evolutionary information content at the expense of logical consistency. We do not regard it as justified to subordinate all other levels of information on organisms—from molecular, karyological, genomic, morphological to ecological—under the principle of a strictly logical ordering system, as demanded by cladistics. On the other hand, it is also not desirable to build classifications only around identifiable phenotypes, especially in view of systems biology attempting to integrate genomics, transcriptomics, proteomics, metabolomics, and so forth. All these levels of organismic organizations are a product of descent and shaped by evolution. Phenotypic similarity alone, without regard to descent, gives classifications that may only be informative for the characters that they are based on, and it may allow taxa defined by homoplasious characters, therefore not based on true homology.

This case study has also shown the limitations of classifications that are only based on DNA sequences of a few loci. This can be as uninformative as pure phenetics based on similarity in a few morphological characters. Combining all datasets provides taxa that are based on descent but circumscribed to emphasize overall divergence of morphological and cytological characteristics. Despite the methodological limitations of total evidence approaches, the analysis of combined datasets appears to be powerful to maximize information content. This approach was also useful on higher taxonomic levels in basal eudicots, i.e., for Ranunculales (Wang et al., 2009), where previous descriptive analyses failed to resolve relationships and resulted in different classifications. The taxonomic framework for *Ranunculus* is, of course, still not perfect; it is likely that unresolved branches or weakly supported clades will get better support with the addition of more DNA markers and cytological data. Within the bigger sections, applications of next generation sequencing to collect whole genome or transcriptome data might greatly improve information content for resolving the internal phylogenies of complex sections (e.g., Harrison & Kidner, 2011). The understanding of modification could be greatly improved as well by additional anatomical, embryological, phytochemical, palynological, and other studies, and also by more detailed ecological data. Evolutionary classification will provide a useful framework for such detailed studies as it provides basic information on the modifications of the phenotype.

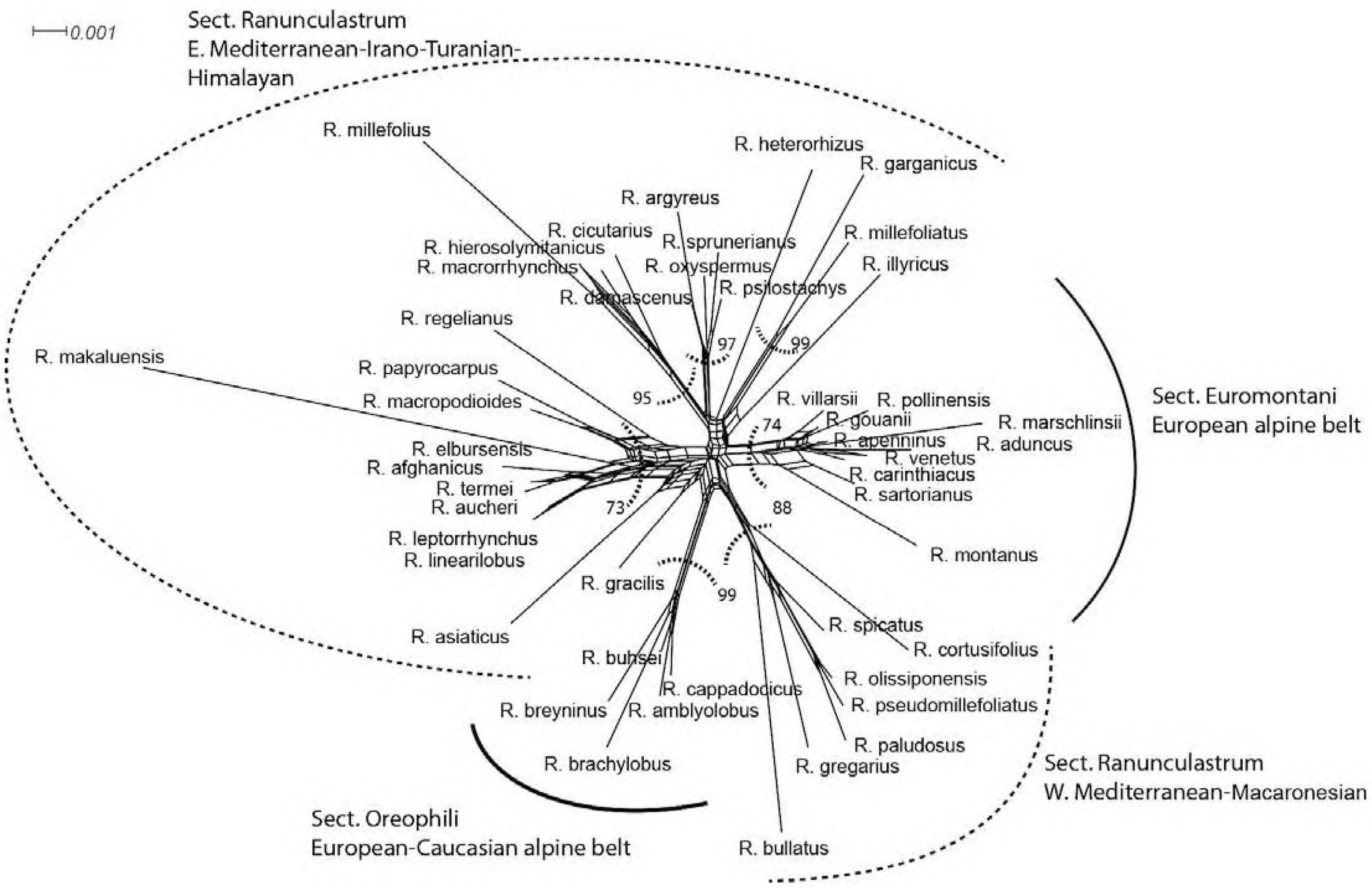


Figure 5. Neighbor-net analysis of clade IX based on the combined molecular dataset (data from Emadzade et al., 2011), using SplitsTree vers. 4.0 (Huson & Bryant, 2006), based on uncorrected p-distances; clusters tested with 1000 bootstrap replicates (values $\geq 50\%$ are shown for main clusters). Scale bar indicates number of changes.

Finally, I would like to address the aspect of information content and practicability. Although many authors rank practicability as the least important criterion (Mishler, 2009), it is an essential feature of information systems to be practicable. For the work of the taxonomist, in fact, the broadening of criteria and datasets effectively reduces the number of equally valid groupings (Fig. 1D). Cladistic classification usually offers many alternative possibilities of selecting groups with shared ancestry (e.g., three in the case of Fig. 1B). Descent alone is a major aspect of evolution, but not a powerful discriminating factor for the definition of biological classes. Phenetics, in contrast, suffers from the many different combinations of character states that produce equally valid patterns of similarity (e.g., four in the case of Fig. 1C). Therefore, phenetics has led to various taxonomic opinions and synonyms, which burdened taxonomy in pre-phylogenetic times. Evolutionary classification has the potential to reduce the number of equally valid groupings: for example, in Figure 1D, only two grouping concepts would be consistent with a criterion of combining phylogeny *and* phenetic similarity: (A, B) and (C, D) or A, B (C, D). In practice, this decision can be based on the degree of divergence case by case. The combination A (B, C, D) and a solution of having four monotypic taxa A, B, C, D are both rejected by phenetic similarities and holophyly of C, D.

We have followed the Linnaean hierarchy for our classification, which of course is artificial, but still widely accepted because of the practicability of ranked hierarchies (Stevens, 2006). The question of appropriate ranking, and the consequence of name changes at different ranks, is not directly resolved by evolutionary classification. In fact, paraphyly could be accepted at different taxonomic ranks: one might follow in *Ranunculus* a concept to classify *Batrachium* or *Ranunculastrum* as genera, rendering the rest of *Ranunculus* paraphyletic. In fact, *Ranunculus* sect. *Batrachium* is the only section that has a unique synapomorphy within the genus. For genera of Ranunculeae, the presence of syn- or automorphies was regarded as an important criterion (Emadzade et al., 2010). However, we considered evolutionary divergence to decide upon the appropriate rank. We did not conduct a patrocladistic analysis (Stuessy & König, 2008); however, the branch lengths of the phylogram of *Ranunculus* and related genera already show that divergence of *Batrachium* from its relatives is lower than divergence among genera (Fig. 2). For this reason, we regard it as more appropriate to keep these paraphyletic groups on the sectional level rather than the generic level.

Evolutionary classification will finally meet the needs of users. In my opinion, taxonomy should not dogmatically override the needs of users, but rather aim at classifications that maximize information for them. In this respect, evolutionary classification can also try to keep the link to traditional classifications and to older literature, where considerable information on phenotype, biogeography, and ecology is being stored. For instance, the name "*Batrachium*" is widely used at various ranks (genus, subgenus, section) in floras and monographs (see surveys in Hörandl et al., 2005; Emadzade et al., 2010). Because of its distinctive features, the circumscription of the taxon has been quite stable in the literature. For the term *Batrachium*, ISI Web of Science lists 56 papers; a Google Scholar search finds 2950 results, and a Google search finds 54,600 results (accessed in April 2012). It would weaken our information systems considerably if we were to sink this taxon into clade III, simply because clade III as a whole has a well-supported hypothesis of a common ancestor. Clade III has never been recognized in this circumscription in the literature, and it is not a class with information content. *Ranunculus* sect. *Hecatonia* does have information content of shared features (see Fig. 2), which differentiates the group from *Ranunculus* sect. *Batrachium*. In this respect, the paraphyletic section *Hecatonia* preserves a well-defined class. Merging sections *Hecatonia* and *Batrachium* would result in a quite heterogeneous group, which would be ecologically and morphologically distinct from *Ranunculus* sect. *Pseudadonis*, but would lose many morphological characteristics. In contrast, *Ranunculus* sect. *Batrachium* is definitely a class, i.e., an information unit; however, this class can be preserved only by breaking with strict inclusiveness and accepting a paraphyletic *Ranunculus* sect. *Hecatonia*, thus recognizing the evolutionary process within the clade. It is necessary to understand how previous literature and modern information systems are linked to worldwide data collection and databasing to document biodiversity, to understand community ecology, species invasions, and extinctions, and to develop conservation strategies. It is further needed for agriculture, forestry, and plant breeding, where traditional literature is still widely used (e.g., Brickell et al., 2008). Modern biology requires more information on taxa than a hypothesis of descent.

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THE CASE AGAINST THE TRANSFER OF *DRYANDRA* TO *BANKSIA* (PROTEACEAE)[1]

Alex S. George[2]

ABSTRACT

The transfer of *Dryandra* R. Br. to *Banksia* L. f. was based on the use of holophyly (monophyly s. str.) as an essential criterion for recognition of taxa. The transfer was significant in scope and focuses on two iconic genera of plants in Western Australia. It has been accepted by some and rejected by others. It is one of many examples in a debate that pits recent genetic analysis against centuries of field and herbarium studies, and cladists against classical taxonomists. I argue that: (1) there are sound morphological characters distinguishing *Dryandra* from *Banksia* and they should be maintained as genera; (2) paraphyly should be accepted in biological classification; (3) scientifically, and for a morphologically complex genus of 137 specific and infraspecific taxa, the use of 11 taxa for the molecular analysis of *Dryandra* was insufficient; (4) some morphological data, mapped onto the cladogram a posteriori, were incorrect; (5) molecular cladistic approaches should complement rather than override pre-existing and extensive classifications based on phenotypic traits; (6) the acceptance of the transfer for the Australian Plant Census was premature according to guidelines published by Australian herbaria.

Key words: Australian Plant Census, *Banksia*, cladistics, *Dryandra*, molecular analyses, paraphyly, Proteaceae.

Over the last four decades, cladistics has become a widely used tool in plant systematics, while molecular analyses have been used increasingly since the early 1990s. Genetic data have become a particularly powerful tool that has enlivened the field of systematics, especially in phylogenetic analyses. However, attempts to reconcile cladistic and traditional classifications have often been controversial (Hörandl & Stuessy, 2010). The purpose of this paper is to review and critique the application of molecular phylogenetic approaches to the systematics of two Australian genera of Proteaceae, *Banksia* L. f. and *Dryandra* R. Br. The intent is to demonstrate that the transfer of *Dryandra* to *Banksia*, which was based on strict adherence to the debatable cladistic concept of holophyly (monophyly s. str.), was not well grounded scientifically, has led to a loss of information, caused confusion among the users of plant names, and joins similarly based transfers of genera in other families. Examples of problems caused by over-reliance on cladistic approaches in plants and animals, especially involving molecular phylogenetic analyses, are used to pinpoint the issues involved in reclassifying life forms based on holophyly. In this case, and in others discussed here, while the data are claimed to show that *Dryandra* makes *Banksia* paraphyletic, the evidence presented for the holophyly of the two genera when combined is based on insufficient sampling.

Stuessy (2009a) and Hörandl and Stuessy (2010), among others, have discussed the different concepts of monophyletic—the original meaning of Haeckel (1866) to define simply a group with a common ancestor, contrasting with the narrow cladistic concept of Hennig (1966) to define a group that includes all the descendants of a common ancestor. The latter has been given another term, "holophyletic," first suggested by Ashlock (1971). The term is used in this paper except in quotes where monophyly is retained rather than assume the meaning intended by the author(s).

In 2007, Mast and Thiele published a paper combining *Dryandra* with *Banksia*, with a further paper later that year covering names that were overlooked in the first paper (Thiele & Mast, 2007). The transfer was based on phylogenetic analyses of molecular (genetic) data. The Australian herbaria quickly adopted the change for the Australian Plant Census, but both the transfer and its rapid acceptance were met with resistance from many users of plant names. Was such a wholesale change justified and necessary? The merger certainly ran counter to conclusions reached in my research of over 50 years in the field, in the herbarium, in horticulture, and in the literature (e.g., George, 1981, 1984, 1996, 1999a, 1999b; Collins et al., 2008). Morphologically, the two genera are readily distinguished and recognizable, although I have deliberated whether certain small groups within each genus should be recognized at

[1] I am grateful to many colleagues, users of plant names, and referees for discussions and critical but constructive comments, but the conclusions and views presented are my own.

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generic rank, in particular *Banksia* subg. *Isostylis* and *Dryandra* subg. *Hemiclidia* and *Dryandra* subg. *Diplophragma*. Disagreement and dismay at the merger from many sources in the community prompted Thiele to publish several popular articles seeking to explain this change to a wider audience (e.g., Thiele, 2008a, 2008b, 2008c).

When Nomenclature Is Changed

The International Code of Nomenclature for algae, fungi, and plants (ICN; McNeill et al., 2012) and its provisions are clear—nobody is obliged to follow a change simply because it is the latest published or because certain institutions have adopted it (George, 2008; see also below). Under the ICN, taxonomic names of plants are available for use if they meet certain criteria, and, if a plant has more than one available name, no further direction is given on how to choose which binomial to use. In the case at hand, the names of all except one species of *Dryandra* meet the criteria, and users can choose whichever generic name they prefer. The exception is a taxon named as *Banksia recurvistylis* K. R. Thiele (Thiele, 2009), for which I present a new combination as a subspecies in *Dryandra* elsewhere (George, 2012). Likewise, there is no mandatory requirement to follow the Australian Plant Census, coordinated by the major Australian herbaria.

The Australian Native Plants Society (formerly Association of Societies for Growing Australian Plants), with several thousand members, has a number of study groups for those interested in particular genera or groups. Among these are the Dryandra Study Group and the Banksia Study Group. The merger of the two genera has been debated in their newsletters (e.g., Cavanagh, 2008a, 2008b; Barrow, 2009) and they continue to accept both genera in the traditional sense. Under the ICN, this is perfectly acceptable, but we have the awkward situation of the "custodians" of plant nomenclature in Australia—some major herbaria—taking one stand, and the users another (including the Catalogue of Plants, Botanic Gardens of Adelaide, 2010). It is apparent that this dichotomy cannot continue indefinitely.

Dryandra and *Banksia*

Dryandra and *Banksia* comprise subtribe Banksiinae of tribe Banksiae, subfamily Grevilleoideae, Proteaceae. Currently, *Banksia* s. str. contains 78 species (with 20 infraspecific taxa) placed in two subgenera, one with 75 species in three sections and 13 series, the other with just three species (George, 1999a; Collins et al., 2008). Most (61) species are endemic in southwestern Australia; 16 species occur in moister areas of eastern and southeastern Australia; and one species occurs across tropical Australia and in New Guinea. *Dryandra* contains 94 species (with 43 infraspecific taxa) in three subgenera (George, 1999b; Cavanagh & Pieroni, 2006). As in *Banksia*, one subgenus contains the majority of the species, 91 placed in 24 series, the other subgenera having two and one species. *Dryandra* is endemic in southwestern Australia.

Two apomorphic states are clear cut in distinguishing *Dryandra* from *Banksia*. First, the receptacle in *Dryandra* is flat or only slightly convex or concave; in *Banksia* it is ovoid to cylindrical. Second, the common and floral bracts (not to be confused with the involucral bracts subtending the whole inflorescence) in *Dryandra* are openly arranged; in *Banksia* they are tightly packed—this distinction is easy to see but it has not been quantified. Thiele and Ladiges (1996: 665) referred to it, as did George (1999b). It has not been mentioned in any of the papers supporting the transfer of *Dryandra* to *Banksia*. Further characters are useful but are not wholly inclusive in distinguishing *Dryandra*: conspicuous, persistent involucral bracts; common and floral bracts that enlarge as the fruit develops; thinner follicles, in many species easily detached. Figure 1 shows the type species of each genus.

In his popular presentations, Thiele has used two diagrammatic "trees" to explain the transfer (Thiele, 2008b, 2008c). One (his fig. 1 in both papers) shows *Banksia* and *Dryandra* arising (evolving) from the same point and is said by him to be the traditional view. The other (his fig. 2) shows *Dryandra* arising from within *Banksia* and is said to be a new result, giving a new understanding, that justifies having to place *Dryandra* in *Banksia*. In fact, the latter "tree" is the traditional view of the relationship of *Dryandra* and *Banksia*. It confirms the view that systematic botanists have held since Robert Brown published the name of the genus *Dryandra* in 1810—to place the genus at the pinnacle of an assumed developmental or evolutionary line within Australian Proteaceae with the implication that, in modern terms, it probably arose out of *Banksia* and is the most highly evolved genus. In other words, contrary to Thiele's claim, the "new" tree confirms the traditional view.

Aspects of the Molecular Work on *Dryandra* and *Banksia*

Data on the material sampled for the molecular analyses are given for Mast and Givnish (2002) on the website of the *American Journal of Botany* at <http://ajbsupp.botany.org/v89/mast.doc> (accessed 2 April



Figure 1. A, B. *Dryandra formosa* R. Br., the type species of *Dryandra*. —A. Headlike inflorescence (photograph: A. George). —B. Infructescence showing involucral bracts and follicles just beginning to open (photograph: A. Cavanagh). C, D. *Banksia serrata* L. f., the type species of *Banksia*. —C. Spikelike inflorescence (photograph: A. George). —D. Infructescence showing two upper follicles still closed, the others opened, and persistent old, unfertilized flowers (photograph: M. Pieroni).

2010). There we find that cultivated material with no vouchers cited was used for four taxa (*Banksia ornata*, *B. penicillata*, *B. saxicola*, *B. spinulosa* var. *neoanglica*), and no source or voucher is cited for a further six taxa (*B. gardneri* [variety not stated], *B. laevigata* subsp. *fuscolutea*, *B. conferta*, *B. paludosa* [variety not stated], *B. ericifolia* "var." [correctly subsp.] *macrantha*, *B. meisneri* "var." [correctly subsp.] *meisneri*), so the determination of these taxa cannot be verified and no repeat of the sampling can be made. They can be reanalyzed only with new samples that may not be identical genetically. The later papers are based on the same samples with several cited additions (Mast et al., 2005: 79). Interestingly, Mast and Thiele (2007) mentioned *Dryandra subulata* as an exception to the holophyly of *Dryandra* because it fell "within a clade of *Banksia* in the cpDNA phylogeny," but the species is not shown in any of the analyses and no voucher is cited.

In transferring *Dryandra* to *Banksia*, Mast and Thiele (2007) stated that "molecular and morphological characters provide evidence that *Banksia* is

paraphyletic with respect to *Dryandra*" and referred to three earlier papers. In the first paper, Mast (1998) sampled the entire internal transcribed spacer region of the nuclear ribosomal DNA (ITS) from 18 taxa of *Banksia* and five of *Dryandra*. He also sampled the *trnL/trnF* region from two non-coding chloroplast DNA (cpDNA) regions from 10 taxa of *Banksia* and four taxa of *Dryandra* (in all papers the two genera in subtribe Musgraveinae were also sampled). In the second paper, Mast and Givnish (2002: 1312) expanded the circumscription of the cpDNA to "chloroplast *trnL* intron, *trnL 3′* exon, and *trnL/trnF* spacer." They sampled 84 taxa of *Banksia* and five taxa of *Dryandra* . . . "for each of these taxa, two to five cpDNA regions were sampled depending on each taxon's function in four sampling strategies" (p. 1312). The regions were *trnL* intron, *trnL/trnF* spacer, *trnT/trnL* spacer, *rp116* intron, and *psbA/trnH* spacer. For the third survey, Mast et al. (2005) sampled 29 taxa of *Banksia* and eight taxa of *Dryandra* and added data from two coding regions, the *matK* and *atpB* chloroplast genes, and the nuclear *waxy* gene. They used the same collections as in the previous research, with the addition of three new collections of *Dryandra* (cited in the paper). Descriptions of the DNA sequence alignments were summarized in Table 2 of Mast et al. (2005: 82).

The sampling of *Banksia* appears adequate (i.e., 84 of 98 taxa). In contrast, only a small proportion of *Dryandra* taxa was sampled. Three taxa of *Dryandra* sampled in the first paper were not used again, and all those in the second were also used in the third. Hence the total number from this genus sampled was 11. Mast et al. (2005: 85–86) considered it an adequate number for their deliberations on the generic status because it represented the three subgenera of the genus, but it is an inadequate number on which to base far-reaching decisions on where to place individual taxa within a generic schema (i.e., transferring taxa to another genus without studying them). As shown by the large number of series, *Dryandra* is highly complex morphologically, so, until a more comprehensive survey is done, we do not know if or how this uneven representation affected the results. Mast and Thiele (2007: 63) referred to "wider sampling of all subgenera and series [of *Dryandra*] except the monotypic series *Decurrentes*" but chose to exclude the data from their paper.

Further questions arise. In Mast and Givnish (2002), all the species analyzed to that time were included in figures 3 and 6 and there are 12 unresolved groups. In Mast et al. (2005), why do none of the trees show all the species of *Banksia* that were analyzed for this and earlier papers? How were the species included in the trees chosen? Did the omitted species show different or anomalous results?

The question of sample size is of great importance, and there is no doubt that the DNA chosen for these analyses represents a small fraction of the total. As Stuessy (2009a: 278) has written, regarding ITS sequences, "If only one sample per taxon is analyzed, the results can be misleading [the same may be said for any character or state used in a systematic study]. The best defense is to sequence three or more accessions per taxon to look for possible multiple copies of the gene." In their early work on *Banksia* and *Dryandra*, Mast and Givnish (2002) "did not sample ITS data at their finer taxonomic sampling because divergent ITS paralogues were discovered among the perhaps thousands of copies of this region . . . and the paralogues from each individual were not always resolved as monophyletic in the phylogenies" (Mast et al., 2005: 79). Mast et al. (2005) therefore extended their sampling to include the waxy gene. Nonetheless, the analyses were presumably based on single samples of each taxon, from the citation of the taxa studied and the vouchers listed (Mast & Givnish, 2002). Despite their uncompromising reliance on holophyly, Edwards et al. (2010, figs. 2 [e.g., AT(1), AT(3)] and 4 [e.g., MH325, GBfoli]) used several samples determined as the same species in their analyses and in several instances these came out separately within the complex, e.g., *Melaleuca leucadendra* (one sample on a separate subclade from the other two), *M. quinquenervia*, and *M. argentea* (their fig. 2).

Would the results have been the same if the whole genome were analyzed rather than several gene regions? Thiele himself (2008a: 14, footnote) also posed the question "how can we possibly get any meaningful answer from such a tiny sample?" His answer (loc. cit., in the text) was that Mast's analyses of several genetic regions "pointed to the same answer (at least in broad outline)" as his own (Thiele's) analyses of morphological and anatomical characters in *Banksia* (no such analysis for *Dryandra* has yet been published). This is standard practice, when several genetic markers are used to carry out maximum likelihood and maximum parsimony analyses (Krane & Raymer, 2003). Mast and Givnish (2002: 1315) reported that "when the relationships within subtribe Banksiinae are compared on strict consensus trees derived from different strategies, there is no topological conflict." There is conflict, or at least different levels of resolution, in the three trees given in Mast et al. (2005; fig. 3A, B), e.g., in the position of *B. elderiana*, *B. media*, and *B. coccinea*,

and in the relationship of a group including *B. elderiana*, especially between figures 3A and 4. In the ITS analysis there was no resolution at all within */Phanerostomata*. Mast et al. (2005) noted the discrepancy in the branching in their cpDNA and ITS analyses but concentrated on the 100% support for the holophyly of the subtribe Banksiinae. Again, would samples of further species, and more samples per species (especially widely distributed, variable ones such as *D. sessilis* and *B. marginata*), have resolved any of this conflict and given more confidence in the results? Later, they stated that "exploration of incongruence among the datasets is outside of [sic] the objectives of this paper" (p. 85), even though the third of their objectives was to "discuss how this [DNA sequence evidence] might influence the biological community's treatment of the two genera in the future" (p. 79). I acknowledge that the authors' focus was on the larger picture of overall groupings and how these should be interpreted at the generic level, but I suggest that results showing poor resolution between the various genetic regions should have been investigated further, especially since these are such important genera. Surely, it is not sound taxonomic practice to make nomenclatural changes without studying every species involved.

Interestingly, He et al. (2011) obtained the sequences of Mast and Givnish for five chloroplast DNA regions from GenBank and reanalyzed them to produce a molecular phylogeny. The programs used were not mentioned. The resultant tree shows general agreement with those of Mast et al. but "with better resolution of more recent divergences" (He et al., 2011: 187). The divergence into */Cryptostomata* and */Phanerostomata* remains, as does the grouping of *Dryandra* as a monophyletic group arising out of */Cryptostomata*. There are, however, marked differences in the position of species such as *Banksia attenuata*, *B. tricuspis*, *B. oblongifolia*, and *B. quercifolia*. */Phanerostomata* appears as the same heterogeneous group of taxa. This also suggests that further research and analysis are needed to investigate the relationship of these taxa.

Paraphyly and Holophyly in Classification

Strict proponents of cladistic classification accept only holophyletic taxa and reject paraphyletic taxa, despite a growing number of papers arguing that this is illogical, ignores many morphological characters, and cannot be applied comfortably to a phylogeny. Taken to an extreme, logical conclusion, all flowering plants would be placed in a single genus, or even species (e.g., Brummitt, 2006: 268). While no one would formally propose this, it becomes relevant at smaller scales. For example, Humphreys and Linder (2009: 1066) wrote that "strict adherence to this statement [recognizing larger monophyletic groups] would mean equating the entire subfamily [danthonioid grasses] with . . . *Danthonia* DC. As this would have several practical disadvantages today, including vast nomenclatural disruption, we do not follow this route." In other words, cladistic classification can be incompatible with what we see around us and impractical for a binomial classificatory system. A number of experienced systematists have presented these arguments, and, to my knowledge, no cladist has answered these criticisms satisfactorily (Schmidt-Lebuhn [2012], however, has made a biased attempt in this direction)—for succinct assessments, see, e.g., Brummitt (2006, 2008), Hörandl (2007, 2010), Stuessy (2009b), and Hörandl and Stuessy (2010). Nelson et al. (2003: 296) responded to Brummitt's (2002) challenge to "draw a phylogenetic tree . . . divided fully into Linnaean taxa without being paraphyletic." However, they drew generic rank across their tree at the third level of nodes from the apex to give four similar taxa (genera). This was one node level above the divergence where major character change occurred in Brummitt's tree that led to one very distinctive genus and three branches representing morphologically similar taxa placed in a single genus. That is, they did not allow for a new taxon evolving very differently while the others remained similar, so that the first was recognized as a distinct genus that would be paraphyletic. This was pointed out by Brummitt (2003), and as far as I know, Nelson et al. (2003) have not responded in the literature.

Pfeil and Crisp (2005) also attempted to answer Brummitt's (2002) challenge to counter the charge that accepting only monophyletic (holophyletic) taxa is illogical. They undermined their argument, however, when, in discussing how to "chop up" a tree and referring to Brummitt's (2002: 32) figure 1, modified by the authors as their figure 2, they accepted the cladistic tenet that "taxa are defined by their shared ancestry rather than their conspicuous characters, that is, they should be monophyletic" (p. 52). They also assumed that Brummitt's (2002) suggested genera were "based on artificial groupings of species." They did not explain "artificial," but it appears that they meant paraphyletic groups even if these are distinguished by good morphological characters. Such characters are determined after sound study of the organisms, not arbitrarily as Pfeil and Crisp (2005) claim. Brummitt gave several examples of taxa in which there are good characters

defining morphologically cohesive ("natural") groups that molecular analysis showed to nest one in another, e.g., Cactaceae within Portulacaceae (and not just within Portulacaceae but within one genus thereof, *Talinum*).

The statement by Pfeil and Crisp (2005: 52) that Brummitt (2002) has created the conflict between paraphyletic and monophyletic (holophyletic) classifications is incorrect. Brummitt was describing a conflict that has arisen over some years from the different approaches between taxonomists who take all characters into account and those who rigidly follow the cladistic tenet of holophyly, and disregard characters that are seen as plesiomorphic. Hörandl and Stuessy (2010: 1641–1642) also discussed the controversy and conflict that have arisen from the refusal by many workers to accept paraphyletic groups, writing: "When cladists elected to allow only holophyletic groups in classification, this excluded paraphyletic groups that were accepted by evolutionary workers. This led to conflict with traditional classifications that had frequently accepted paraphyletic taxa because of shared similarity." Another error by Pfeil and Crisp was their attribution (p. 53) of the following statement to Brummitt: "Hennig fatally ignored Darwin's observation ... that evolutionary classification depends on two factors, descent and modification. Hennig ignored modification and thought classification could be based only [word omitted by Pfeil and Crisp] on phylogeny irrespective of characters." In writing this, Brummitt attributed it to Ernst Mayr. Pfeil and Crisp chose not to comment on Brummitt's subsequent statement, that Hennig attempted "to equate the hierarchy of descent directly with the hierarchy of Linnaean classification, but, as I hope I have demonstrated, these are incompatible" (Brummitt, 2002: 35).

Pfeil and Crisp's (2005: 53) conclusion that "the solution to Brummitt's conundrum is to use phylogenetic trees to define higher taxa—not an arbitrary subset of the characters that produced the trees" is no solution at all. It simply confirms their strict adherence to holophyly. They did not address Brummitt's challenge to "draw a phylogenetic tree and divide it into families and genera without creating paraphyletic families and genera" (Brummitt, 2002: 33).

As Brummitt (2006: 269) has written, sinking morphologically distinct taxa into a paraphyletic parental taxon merges them into an "amorphous plesiomorphic soup" and is a denial of evolution—it does not recognize newly evolved taxa. "Since, in the cladist view, no family or genus can have evolved from another one, it is effectively a denial of evolution having taken place. ... To overcome these objections to cladistic taxonomy, its proponents argue that we are dealing only with present-day taxa and must ignore ancestors because we do not have them and so cannot name them. So at the same time as they define paraphyly in terms of ancestry, they insist on ignoring ancestry. This is not logical."

To put it another way: just because a group of taxa is nested in a molecular or cladistic analysis within others is no logical reason to lump them taxonomically. It's a matter of where you draw the line or lines delimiting rank on a tree. In the case of *Banksia* and *Dryandra*, instead of lumping the operational taxonomic units (OTUs) into a single taxon, this could have led to recognition of more taxa and still been concordant with the data. Mast and Thiele did not explain why they did not wish to recognize tribe Banksiinae at generic rank and include *Musgravea* and *Austromuellera*. Two analyses of Mast et al. (2005) show 100% and 92% bootstrap support for their inclusion with *Banksia* and *Dryandra* in a single taxon, and retaining them as separate genera makes them paraphyletic with respect to *Banksia*. Perhaps this is an instance of Pfeil and Crisp's (2005: 52) "but it's so different" (BISD) argument? If more genera were included in any cladistic analysis, those higher on the cladogram might (or almost certainly would) make those lower down paraphyletic, necessitating lumping more genera in order to maintain holophyly. An example of including more genera is a paper by Edwards et al. (2010), in which all nine genera of the tribe Melaleuceae (Myrtaceae) were analyzed and *Melaleuca* found to be paraphyletic, leading them to conclude that all nine must be combined as a single genus. The taxonomic sample was small, 50 species of a total of ca. 335 for the tribe, with 36 species of *Melaleuca* (current total 259) sampled, one of *Calothamnus* (41), three of *Beaufortia* (15), two of *Eremaea* (15), one of *Regelia* (6), two of *Conothamnus* (3), one of *Phymatocarpus* (3), two of *Lamarchea* (2), and one of *Petraeomyrtus* (1). While bootstrap support for the tribe was strong, that for the three clades within it was less so—56%, 81%, and 67% (their fig. 1). Within their clade A (their fig. 2), which contains the genera currently recognized to be distinct from *Melaleuca*, support for some nodes was weak, with a bootstrap value of 51% for the node from which all genera except *Lamarchea* and *Beaufortia* diverge from *Melaleuca*, and support for *Beaufortia* was only 54%. Several species of *Melaleuca* came out in the "other genera" clade. The authors proposed merging all genera in *Melaleuca* because, firstly, "the morphological

characters examined in this study were found to have high levels of homoplasy and to lack diagnostic ability" (Edwards et al., 2010: 753). They did not elaborate on how they came to these conclusions, nor did they say whether they had looked for more than the 27 characters listed in their Appendix 1. Secondly, they considered that "it is desirable to avoid generation of many new genera with few representatives." There is no scientific basis for this view. The proposal to merge these genera appears to be unsound.[1]

A telling comment comes from Cavalier-Smith (2010: 111) who wrote: "Hennigian cladistics emphasizes only lineage splitting, ignoring most other major phylogenetic processes. Though methodologically useful it has been conceptually confusing and harmed taxonomy, especially in mistakenly opposing ancestral (paraphyletic) taxa."

Comments by Grant (2003: 1268) are pertinent:

> Cladistics is based on inferred phylogenies, which makes for an uncertain foundation. Phylogenies of groups above the species level are, with rare exceptions, unverifiable hypotheses. Taxonomic systems are based on observable characters and do not rest on phylogenetic hypotheses, although taxonomists may (and do) weight characters and states differently.
>
> . . . the information base . . . is very broad in taxonomy in which no class of characters is excluded from consideration. It is narrower in morphological cladistics because of the exclusion of certain types of potentially valuable characters, and it is extremely narrow in molecular cladistics. Taxonomy has a more inclusive empirical foundation than cladistics.

Stuessy (2009b: 72) has pointed out that cladistics has put interpretation of characters back into biological classification but at a cost: it focuses solely on branching patterns and reduces the information content. More recently, Stuessy and Hörandl (2010: 1642) have written:

> Hennigian and post-Hennigian cladistics have disregarded evolutionary data for the main purpose of constructing the branching pattern. The data of importance become the synapomorphies, whereas autapomorphies are discarded as noninformative. Emphasis on holophyly in a quantitative method of phylogeny reconstruction has resulted in a narrower (and less informative) approach to biological classification. Cladistic principles stress a restrictive hypothesis, that of dichotomous splitting of a lineage into two new lineages, resulting in extinction of the ancestor. Whether other evolutionary processes (e.g., budding and merging) are involved is neither tested, nor verified, nor falsified, but rather neglected a priori (Hörandl, 2010). Statistical tests of tree topologies, e.g., bootstrapping, support tree topologies under the assumption of dichotomies, but they do not test the assumptions themselves. Cladograms are useful tools to formulate specific hypotheses, but because of their theoretical restriction, they are not sufficient to yield robust evolutionary conclusions.

Pfeil and Crisp's assertion (2005: 53) that "the most recent common ancestor of e.g., a pair of genera, should not logically belong in either descendent genus" (repeated a little later as "the ancestral organism cannot have been a member of either modern genus") is itself not logical. The parent of an offspring that leads to a new taxon may survive for a long time and remains within its original taxon—it doesn't change its position in a classification because it has produced offspring. The line of descent of the original ancestor may continue through one descendant while a new line evolves through another (Hörandl & Stuessy, 2010: 1645).

Pfeil and Crisp (2005: 52) argued that paraphyly "perpetuates a classification that obscures information and reduces predictive power." In the case of *Dryandra* and *Banksia*, the reduction of *Dryandra* to a single series within *Banksia* in order to make the latter holophyletic has created a classification that causes just this loss.

Stace (2005: 1000) also had reservations about monophyly (holophyly), writing: "I personally advocate recognising paraphyletic taxa if this produces the most predictive classification, and in my view an absolute insistence on monophyly whatever the circumstances amounts simply to dogma. A monophyletic system of classification that is not the most predictive available becomes a special purpose classification. I would say that a phylogenetic special-purpose classification is about as practically useful as the party bore who details his troubled journey there to anyone who will listen."

The importance of accepting paraphyletic groups is summarized by Hörandl and Stuessy (2010: 1650):

> Above the species level, retention of paraphyly and holophyly is essential for the theoretical foundation of taxa through natural processes (common ancestry) and hence for predictivity. Information content and practicability, however, are needed as additional criteria to translate natural taxa into a formal classification. Information content and practicability are dependent on human perception, human logic, terminology, and utility of ordering systems of organisms. These anthropocentric aspects are obviously important for classification, as a global information system for organisms must be provided for the scientific community and for society in general. These principles are violated if classifications abandon paraphyletic taxa that are actually monophyletic s.l., although they may not appear as holophyletic clades in

[1] Despite these reservations (in particular the small taxonomic sample), and with no further research, the merger of all genera into *Melaleuca* has now been published (Craven et al., 2014). It is to be hoped that the Australian herbaria will follow their guidelines when assessing this for the Australian Plant Census. [Note added in proof.]

cladograms. Because of shared ancestry, paraphyletic taxa often have high phenotypic similarity and information content.

It is unfortunate that Mast and Thiele and their coworkers have not addressed the problem of paraphyly in their work on *Banksia* and *Dryandra*. Indeed, Mast et al. (2005: 86) wrote that "It is not within the scope of this paper to critically evaluate the place of paraphyletic taxa in taxonomy ...." Given that two major, well-known Australian genera are involved, and that paraphyly is a critical issue (as indicated by inclusion of the term in the title of their paper), it should have been discussed.

Mast and Thiele (2007: 63) united *Dryandra* with *Banksia* because their analyses "showed strong evidence for the paraphyly of *Banksia* with respect to *Dryandra*." They stated that "published and molecular evidence provide strong support for the monophyly [holophyly] of *Dryandra*," but this was based on limited sampling of the taxon. While they had strong support for the group containing both *Dryandra* and *Banksia*, they cannot show that a combined *Dryandra* and *Banksia* is monophyletic (holophyletic) until *Dryandra* is sampled much more extensively.

I support the recognition of paraphyletic taxa in Linnaean classification, and maintain that, given present data, *Dryandra* is an easily recognized, holophyletic taxon that should be accepted at generic rank. *Banksia* s. str., considered paraphyletic by Mast and Thiele, should continue to be accepted pending further work. Whether *Dryandra* is, in fact, holophyletic remains to be shown by wider sampling. This may confirm that it is holophyletic, or show that both it and *Banksia* are polyphyletic. Given the morphological diversity of both genera, it is more likely that holophyly will be maintained only by recognizing more genera. Clearly, further research is needed to resolve the classification of these taxa.

Other Aspects

Thiele (2008b) wrote that, because some species of *Banksia* are related more closely to *Dryandra* than to other banksias, keeping the genera separate is a "serious anomaly." This need not be the case—it's obvious that, when a new organism evolves from a member of a large group, it is going to be related more closely to that member than to the others. At some point it (and its descendants) may then become different enough to be recognized as a new genus. It would have been valuable for Mast and Thiele to take their research further to investigate how best to resolve the three branches on their tree (Mast & Thiele, 2007: 64; see Fig. 1 below) leading to unlabeled parts of */Cryptostomata*. This may indicate which species or group of species is sister to *Dryandra* and show where research should be focused regarding the origin of the latter.

Thiele (2008c: 7) also claimed that their molecular analysis explains the distribution of *Dryandra* in southwestern Australia—it evolved from "its banksia ancestor ... after the south-west was isolated by increasing aridity, and none of its ancestors managed to cross the deserts." In fact, the analysis simply confirms what has been said many times—that many groups of plants and animals have evolved in southwestern Australia since the Nullarbor Plain and deserts formed (e.g., Hopper & Gioia, 2004). The analysis of He et al. (2011) also confirms that *Dryandra* arose after the formation of the Nullarbor Plain. This is an example of loss of information from combining *Dryandra* with *Banksia*: Barker et al. (2007) put the origin of a combined genus at 65 Ma (thus giving no indication for *Dryandra*), and Sauquet et al. (2009) determined it as ca. 50 Ma, whereas He et al. (2011), analyzing the genera separately, confirmed *Banksia* s. str. at the same age but placed the origin of *Dryandra* at ca. 15 Ma.

As support for a major generic change, the phylogeny shown in figure 1 of Mast and Thiele (2007: 64) is remarkably simplistic and misleading. It is said to be "modified from fig. 1b of Mast et al. 2005," but that figure is a photograph of *Dryandra ferruginea* and presumably the reference is to figure 2B (Fig. 2 in this paper). Compared with that figure, Mast and Thiele's figure 1 of 2007 has sprouted a third branch above "beaked follicles," explained with the statement in the caption that "the number [of lineages] is variable in the phylogenies inferred with each of the datasets" (p. 64). In order to find which taxa each branch of these figures leads to, one must try to reconcile them with figures 3 and 4 of Mast et al. (2005) but, because of a different style of branching, that is a difficult task.

On Morphology

Mast and Thiele's claim (2007: 63) that "morphological characters provide evidence that *Banksia* is paraphyletic with respect to *Dryandra*" was overstated, because they did not make a detailed morphological analysis of all taxa of *Dryandra*. Rather, a posteriori they placed a few characters on their molecular trees—see figure 2B of Mast et al. (2005) (Fig. 2 in this paper), given in simplified form as figure 1 in Mast and Thiele (2007). As argued by Assis and Rieppel (2011: 97), synapomorphy and monophyly are not the same thing and mapping

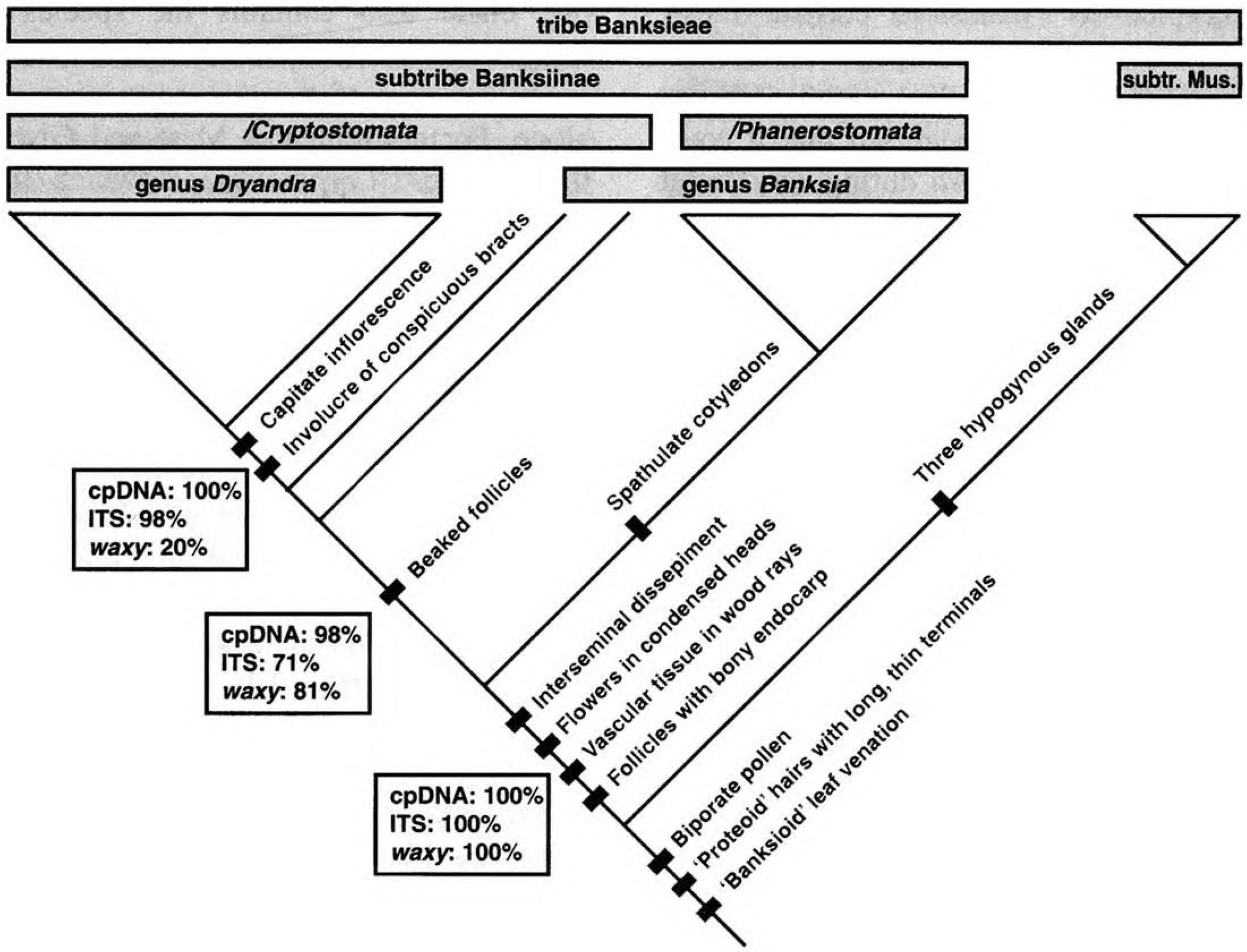


Figure 2. Figure 2B from Mast et al. (2005). Reproduced with permission from *Australian Systematic Botany* 18: 75–88 <http://dx.doi.org/10.1071/SB04015> (Mast et al.) Copyright CSIRO 2005. Published by CSIRO PUBLISHING, Collingwood, Victoria, Australia. Original caption: "Phylogenies reconstructed for members of the tribe [Banksieae] by Mast (1998) and this study using the two internal transcribed spacers of the nuclear ribosomal DNA (ITS), Mast and Givnish (2002) and this study using chloroplast DNA (cpDNA), and this study using the nuclear waxy gene resolve a paraphyletic *Banksia* with respect to *Dryandra*. Bootstrap frequencies for three branches as determined in this study are given in boxes adjacent to the branches. Mast and Givnish (2002) recognised a morphological synapomorphy for each of the clades formed at the basal split of the subtribe in the DNA phylogenies and referred to these as */Cryptostomata* and */Phanerostomata*. Note that 'Subtr. Mus.' is subtribe Musgraveinae, containing two monotypic genera."

This figure was reproduced as figure 1 in Mast and Thiele (2007), but with the lowest branch (leading to subtribe Musgraveinae) deleted, "subtribe Banksiinae" replaced by *Banksia*, the generic names *Dryandra* and *Banksia* deleted, and the names "subgen. *Banksia*" and "subgen. *Spathulata*" added to */Cryptostomata* and */Phanerostomata*, respectively. Also, a third, unresolved branch was added above the character "Beaked follicles" leading to */Cryptostomata*, with a note in the caption that the number of lineages is "variable in the phylogenies inferred with each of the datasets."

morphological characters onto a phylogenetic tree derived from molecular data is an empty procedure, since those characters have not been tested for congruence with the data from which the tree has been built. Further, a useful definition of a synapomorphy is "a shared, derived character state." Far from being character states, those applied here are, rather, complex character suites and, given the relatively large number of species being dealt with, possibly homoplasious, but further research would be needed to confirm or refute this. This aside, there are problems with these "synapomorphies." Below the first branch is shown "Flowers in condensed heads" and then, below the terminal branch leading to *Dryandra*, "Capitate inflorescence." Both phrases mean much the same thing. The lower one should be, say, "Flowers in condensed inflorescences/conflorescences on a woody receptacle." The upper is then correct. On the first branch, leading to */Phanerostomata*, "Spathulate cotyledons" is given as a synapomorphy, but as discussed below in regard to *Banksia* subg. *Spathulatae*, spathulate cotyledons also occur in species of */Cryptostomata* and *Dryandra*. Cotyledon shape in *Banksia* and *Dryandra* is also complex and this mapping onto the tree is a misleading oversimplification. Then, above the first

branch they give "Beaked follicles" leading to the remainder of *Banksia* and all *Dryandra*; but not all species of *Dryandra* have beaked follicles (a note in Mast & Givnish, 2002: 1319, citing information received from me, correctly says just that "*Dryandra* commonly has beaked follicles"). That is, the statement in Mast et al. (2005), repeated in Mast and Thiele (2007), that beaked follicle valves are a morphological synapomorphy supporting the holophyly of */Cryptostomata* is not correct, although the character could have been lost secondarily. Finally, in figure 1 (2007), their "Involucre of conspicuous bracts" as a synapomorphy for *Dryandra* is also incorrect. All species of *Banksia* and all species of *Dryandra* have an involucre of bracts subtending the inflorescence. It is correct that in most species of *Banksia* these are inconspicuous (in fact, in many they fall by anthesis), but in several species such as *B. goodii* and *B. victoriae* the involucre is conspicuous and persistent. Conversely, in most species of *Dryandra* the involucre is conspicuous, but in some it is not, e.g., *D. concinna* and *D. sessilis*. Again, the involucre is a complex character suite.

The new *Banksia* subg. *Spathulatae* proposed for the species in clademark */Phanerostomata* is distinguished in the diagnosis thus: "Differing from all other species of *Banksia* in having spathulate cotyledons" (Mast & Thiele, 2007: 65). As just stated, spathulate cotyledons occur in a number of species of *Banksia*, not just those in */Phanerostomata* and they also occur in *Dryandra*—they may be broad or narrow, but they are still spathulate. Hence, Mast and Thiele's diagnosis of their *Banksia* subg. *Spathulatae* is imprecise, though, strictly speaking, there is nothing in the ICN to invalidate it. Their reference under the etymology of the name to "distinctively spathulate cotyledons" without further elaboration indicates an incomplete survey of *Dryandra* and *Banksia*.

Further, the only indication of the taxa that they include in *Banksia* subg. *Spathulatae* (besides the type, *B. integrifolia* L. f.) is a statement in the text on p. 64: "we establish *Banksia* subgen. *Spathulatae* for the species in */Phanerostomata*." There is no reference to where a circumscription of this "clademark" and the taxa included may be found. From mentions of */Phanerostomata* earlier in the paper, one can assume that it is to be found in Mast and Givnish (2002) and/or Mast et al. (2005). The latter includes only nine species of */Phanerostomata* and 20 of */Cryptostomata*, thus not listing some 50 species of *Banksia* s. str. The former lists all species in figures 3, 4, and 6, but figure 6 also includes *Austromuellera trinervia* and *Musgravea heterophylla* in */Phanerostomata*. Presumably the two last taxa are excluded from *Banksia* subg. *Spathulatae* but we are not told this explicitly. From a systematic angle this means that we cannot assign unsampled taxa to these clademarks. Furthermore, the species included in */Phanerostomata* sensu Mast and Givnish (2002) are a very disparate group morphologically. They include some but not all of the "straight-styled" species of section *Banksia* and all the "hook-styled" species of section *Oncostylis*, very distinct sections morphologically and accepted by all since the work of Bentham (1870). In Thiele and Ladiges (1996), they were placed in six series of subgenus *Banksia* (they did not recognize sections). Several infrageneric taxa have been heterogeneous in all generic treatments, but section *Banksia* and section *Oncostylis* remain well defined, so the distribution of their species between */Phanerostomata* and */Cryptostomata* should be investigated further. If the clademark is an evolutionary phenomenon, then there must be character states, however cryptic, that link the included species as a group. More discussion of this would have been informative and, if soundly based, would give more confidence in the reality of *Banksia* subg. *Spathulatae*.

Note, too, that */Phanerostomata* is defined as "typically tall shrubs and trees that occupy moister coastal mountains, especially in eastern Australia, and produce short-lived leaves that are thin, soft and less strongly toothed" (Mast & Givnish, 2002: 1322); but some species included by them do not meet these criteria: e.g., *Banksia nutans*, *B. scabrella*, *B. sphaerocarpa*, *B. grossa*, and *B. incana*, which have tough leaves and occur in Western Australia in open heathlands with relatively low rainfall. Incidentally, in describing the arrangement of the stomata in *Banksia*, Thiele and Ladiges (1996: 669) wrote that "In series *Salicinae*, *Grandes* and *Coccineae*, the pits are relatively indistinct, large, wide and shallow depressions, while in the remaining species they are smaller, more definite, and often crypt-like, with constricted entrances. However, there is a continuous grade between these pit types." This is not the clear distinction implied by Mast and Givnish (2002) in their clademarks. In including *Dryandra* in their concept of */Cryptostomata*, Mast and Thiele (2007) have not said whether they surveyed this character in all taxa of *Dryandra*. Since species of *Dryandra* grow in habitats ranging from forest with relatively high rainfall to inland shrublands with low rainfall, and their leaves range from relatively soft to hard (with or without revolute margins), it is likely that not all belong with the concept of stomatal arrangement in */Cryptostomata*—another area of insufficient research in their work.

A detailed morphological analysis of *Dryandra* (similar to that of Thiele and Ladiges for *Banksia*) is still awaited. For their morphological data on *Dryandra*, Mast and coauthors presumably relied on descriptive work published by previous workers, rather than study the plants to assess whether the molecular data are supported by the morphology. Mast et al. (2005: 86) acknowledged that "we do not have the morphological data that might help to place it [*Dryandra*] when analysed in concert with that sampled in *Banksia* by Thiele and Ladiges (1996)." Further, Mast and Thiele (2007: 65) acknowledged that their results are preliminary, stating that their new classification "is the least disruptive option at present." They referred to "fine-scale taxonomic sampling" being carried out in Mast's laboratory, without explaining what they meant by this. If it is a full morphological analysis of *Dryandra* then this should have been completed before making sweeping nomenclatural changes.

Furthermore...

Mast and Thiele's claim (2007: 64) that an expanded *Banksia* is "a single, easily recognised genus equivalent to the subtribe Banksiinae" is debatable. Separately, *Banksia* and *Dryandra* are readily distinguished morphologically—even people with just basic training have no difficulty recognizing a *Banksia* or a *Dryandra*, even if it is a species that they have not seen before. In fact, combining the two creates a less clearly definable taxon and implies that these authors do not know their plants well. They have not provided an amended description of their *Banksia* s.l., or a comprehensive infrageneric table (see below), or a key to enable people to identify specimens, or even specifically cited works where keys are to be found. Their "automatic" transfer of all taxa recognized by me in *Dryandra* means that they made no reassessment of those taxa. In a complex genus of great morphological diversity, and with new material being added to collections, it is almost certain that a thorough revision would result in changes.

It is noteworthy that Thiele and Ladiges (1996) were unable to determine an outgroup for comparison of *Banksia* and placed subgenus *Isostylis* and subgenus *Banksia* as sister taxa, so that *Isostylis* was basal in their cladograms. The molecular analyses of Mast et al. have consistently placed *Isostylis* as the most advanced group within *Banksia*, as indeed it was in my systematic sequence (George, 1981, 1999a).

Mast et al. (2005: 86) wrote that combining *Dryandra* with *Banksia* "would make *Banksia* the seventh largest genus of vascular plants in Australia." Is this supposed to make us feel good about the merger? They also claimed that it "would probably confer greater research attention on the species of *Dryandra*," and that their inclusion in any comparative study with "exhaustive" taxonomic sampling of *Banksia* "would become certain." These are not scientific arguments, and one can just as easily argue the opposite—that submerging one genus in another (especially at a low infrageneric rank) makes its taxa less conspicuous (Hörandl & Stuessy, 2010: 1647). As stated above, Mast and Thiele should themselves have done far more research before drawing taxonomic conclusions. While this might contribute to our understanding of these fascinating plants, there are many organisms that we hardly know, that are unclassified, to which future direction of such considerable expertise and resources would contribute much more to our knowledge of the world's biodiversity.

Finally, as Winston Churchill might have said, there is some "terminological inexactitude" in the nomenclatural changes of Mast and Thiele. The name *Banksia* ser. *Dryandra* is not just a "comb. nov." but also a "stat. nov." For taxa in *Dryandra* with epithets already occupied in *Banksia*, the designation "nom. nov." is only half the story, as they are also "comb. nov." Also, their use of "basionym" for the replaced names is incorrect—they are not basionyms but replaced synonyms. Several of their new epithets are inappropriate, for example, *obovata* to replace *cuneata* "in reference to the obovate leaf shape" (Mast & Thiele, 2007: 65), when the leaves are typically cuneate, only occasionally obovate; *pellaeifolia* to replace *blechnifolia* "from *Pellaea* a genus of pinnate ferns and *folium* (a leaf), in reference to the divided, fern-like leaves" (no species of *Dryandra* has pinnate leaves).

Infrageneric Classification

Mast and Thiele (2007) have provided minimal data on an infrageneric classification of their expanded *Banksia*. From their figure 1 (cf. Fig. 2 above) we know that they recognize two subgenera, *Banksia* subg. *Banksia* and *Banksia* subg. *Spathulatae*, and one series, *Dryandra*. We can deduce that they recognize the autonym series *Banksia* in subgenus *Banksia*. Beyond that, nothing. They have not said whether they recognize *Banksia* subg. *Isostylis* R. Br. or, if not, which of their subgenera they place it in and at what rank. A phylogenetic tree should reflect the classification of the taxon or taxa concerned, but this is impossible from Mast and Thiele's figure 1, the only figure presented, and which gives only "the major features of the phylogeny."

Their new classification obscures relationships because they have placed all 94 species of *Dryandra* in a single series within *Banksia* while (presumably but not stated explicitly) retaining a comprehensive infrageneric classification for the taxa of *Banksia* s. str. They stated, on p. 64, that the infrageneric classification of *Banksia* "has been tested against phylogenies generated from morphological and molecular data" but did not say which classification they tested or what the results of that testing were. Their subgenus *Spathulatae* contains both straight- and hooked-styled species, but we are left in the dark as to which those species are, and indeed, what infrageneric classification they accept for taxa of *Banksia* s. str. in their expanded genus. Further, with three subgenera and 24 series, *Dryandra* s. str. is at least as diverse as *Banksia*, and this should be reflected in its classification even if it is called *Banksia*. When *Dryandra* is ranked as a series in Mast and Thiele's classification all this information is lost, i.e., their expanded *Banksia* loses predictability.

All we can deduce from their paper about an infrageneric classification is the following:

Banksia
subgenus *Banksia*
series *Banksia* (type, *B. serrata*)
series *Dryandra* (type not stated, presumably *B. formosa*)
subgenus *Spathulatae* (type, *B. integrifolia*)

In other words, while inferences may be made from their background papers, we can place just three of some 172 species in their classificatory scheme with certainty.

Taxonomy in the Face of Uncertainty

Reliance on phylogenetic and cladistic analyses is called into question when one considers the uncertainties and assumptions in many published papers (not to mention the assumptions that lie behind all the computer programs used). As discussed elsewhere in this paper, authors themselves often raise doubts about their results through frequent use of words such as "may/might," "suggest," "appear," and "seem" but make nomenclatural changes regardless. We also have the situation of the same author being involved in the transfer of species from one genus to another (Crisp & Weston, 1987) and then back again a few years later after further research (Chandler et al., 2002), or revising a genus (Crisp, 1995) and then transferring it to another just seven years later (Chandler et al., 2002). The last paper transferred three genera to *Gastrolobium* because their analyses "showed that *Gastrolobium* is paraphyletic" (Chandler et al., 2002: 622) but with no further discussion. Lewis et al. (2005: 4–10), in the section "Changes in generic alignment and tribal composition in the legumes since Polhill (1994)" discussed a number of examples of changes, "reversals," and uncertainties in tribal and generic placement. Crisp and coauthors, and Mast and Thiele, should have done as others faced with inconclusive results have done and undertaken or awaited further research, as was the case for a study of the Chloantheae by Conn et al. (2009: 255) who concluded: "To ensure nomenclatural stability, we recommend that a more comprehensive sample from each of the genera be analysed before formal changes are made." Nonetheless, without further sampling, Conn et al. (2011) presented a new generic classification within the tribe, with associated nomenclatural changes.

A recent paper on *Callitris* and *Actinostrobus* (Piggin & Bruhl, 2010) is a further example of making nomenclatural changes in the face of uncertainty and inadequate assessment of data, in part by the authors being inconsistent in following their own criteria. They (p. 72) regarded bootstrap values of 50%–70% as weakly supported and 70%–90% as moderately supported yet, in their figure 1, six branches had values within the lower range and a further eight were in the moderate range. One branch was below 50% (that leading to Venning's "complex"), and only a single branch (leading to *Actinostrobus*) had strong support (94%). The consistency and retention indexes were also low, at 0.45 and 0.74, respectively. The three species of *Actinostrobus* came out as a robust, holophyletic group in each of their analyses. They listed (p. 74) seven morphological features that distinguish *Actinostrobus* from *Callitris*, although the most distinctive, the presence of sterile scales at the base of female cones, was excluded (without explanation) from their character analysis. Piggin and Bruhl were not confident of their conclusion, stating that "further molecular data are needed to test these results" (2010: abstract, 69). While their data appear to show that *Actinostrobus* makes *Callitris* paraphyletic, they have not shown that the two taxa, when combined, form a holophyletic genus. Yet, with no discussion beyond describing the defence of paraphyletic taxa as "ad hoc," Piggin and Bruhl combined the two genera. Their claim (p. 78) that including *Actinostrobus* within *Callitris* "goes some way towards resolving the instability in [taxonomy of] *Callitris*" appears to mistake where that problem lies—they stated later (p. 80) that "Instability in *Callitris* taxonomy has centred on how to best deal with the seven or eight taxa that make up

Venning's (1979) complex" of *C. glaucophylla* and allies. As all their analyses show, *Actinostrobus* has nothing to do with this group. And, by combining *Actinostrobus* with *Callitris* and not erecting any infrageneric taxon for it, information is lost. The question must be asked: given this uncertainty, why publish these nomenclatural changes? Once again, a refusal to accept paraphyletic taxa has led to an arguably unnecessary combination of genera.

I do not suggest that we await certainty before publishing. If we adopted that approach we would get nowhere. But we should have a firm foundation on which to base major changes in classification and nomenclature, especially those of long standing. Writing about phylogenetic studies, Orthia et al. (2005: 46) wrote, "Studies that do not thoroughly explore the evolutionary history of the study group . . . might be making erroneous or premature judgments about taxonomic boundaries that ultimately perpetuate instability."

Objectivity

Molecular and cladistic analyses are promoted as objective, but many subjective decisions are made in these methodologies (reviewed by Stuessy, 2009a). A critical point is the lack of any objective criterion or measure for determining taxonomic rank in molecular analyses—whether it's flowering plants, bryophytes, reptiles, or fish, the worker makes a subjective assessment of "what looks right." Such analyses are also claimed to be repeatable (and by implication reliable), since the data are available to other researchers at GenBank and analysis of the data can be rerun. But can we readily resample the vouchers for new genetic samples in order to compare analyses (see above for comments on the vouchers used in *Dryandra* and *Banksia*)? Material from other plants of the same species may not give similar results (e.g., Edwards et al., 2010, see above), but unless multiple sampling (both of species and within species) is done, then I contend that the results should be considered preliminary. Although the molecular research program on *Banksia* and *Dryandra* has been running for some 15 years, the results obtained so far are not sufficiently robust to justify major taxonomic changes. The study of morphology is just as repeatable, and we can look at multiple specimens, some the same that previous botanists looked at—such as (in the case of Proteaceae) Robert Brown (1810), Carl Meisner (1856), and George Bentham (1870) in the 19th century, Lawrie Johnson and Barbara Briggs (1975) and others in the 20th.

Mast and Thiele (2007) made a subjective decision on what course to take in resolving their taxonomic problem. Since, according to their cladistic interpretation, they could not maintain the status quo, they considered three options, each of which had varying degrees of support in their analyses, but these levels of support did not enter into their final decision. They discarded their option two (to maintain *Dryandra* and split *Banksia*) because it would require the "establishment of several new, less distinctive genera" (p. 64). This is a subjective decision that we cannot assess because we are not told which species would be placed in which genus and, therefore, cannot consider how distinctive the new genera would be. If they correspond to any of the infrageneric taxa in my classification (George, 1999a), then there are good characters to distinguish them. They discarded option three (to establish two genera equivalent to the clades formed at the basal split of *Banksia*, i.e., */Cryptostomata* and */Phanerostomata*) because it "requires the most complicated nomenclatural changes" (Mast & Thiele, 2007: 64), a reason I have seen no other researcher offer for not proceeding with amending a classification, and again, a subjective decision. Clearly, Mast and Thiele were prepared to consider recognizing multiple genera on less than 100% support, but, in effect, they took the easy way out by adopting their option one, to reduce *Dryandra* to a synonym of *Banksia*.

The Concept of the Genus

The question of generic delimitation and size has occupied a number of authors. For example, Pfeil and Crisp (2005), in a section entitled "Is a genus real, and if so, in what sense?," stated that their concept of genera is "groups of organisms more inclusive than a single species." In a paper in the same number of *Australian Systematic Botany*, however, Orthia et al. (2005) appear to accept monotypic genera such as *Euchilopsis*, stating (p. 43) that "there is nothing wrong in principle with the circumscription of small or monotypic genera" but later (p. 46) indicate that they would have a problem with many small genera that had "the same status" as large genera. They felt that splitting *Pultenaea* s.l. into multiple genera, as their cladistic analysis indicates, "would introduce greater taxonomic inequivalence, maintain instability and enforce monophyly onto reticulate groups within it" (p. 46). As mentioned above, Edwards et al. (2010) also had problems with small genera. As Hörandl (2010: 349) has pointed out, however, "If rapid morphological divergence occurs in single

lineages, then a classification of monotypic genera is more informative about this divergence than classifying a heterogeneous clade as a taxon." There is nothing scientifically wrong with the concept of small genera. Almost certainly, every genus in its beginning was monotypic.

As mentioned above, Humphreys and Linder (2009), in their work on danthonioid grasses, decided not to adhere to the concept of larger monophyletic (holophyletic) genera over several small ones since it would have practical disadvantages. They wrote (p. 1054):

> "Stability" increases communication power of a classification by perpetuated use of already accepted names . . . and is maintained by delimitation of genera in such a way that future nomenclatural changes will be unnecessary. . . . efforts to define a genus that reflects evolutionary patterns should not override those that ensure its usefulness. Consequently, monophyly is important only in so far as it increases the chances of a genus being both stable and predictive.

As Nyffeler and Eggli have written (2010: 237), "Discordance between molecular phylogenies and traditional classification practice should rather be seen as a challenge to further investigate morphological characteristics potentially useful for circumscribing and identifying taxa derived from inferred phylogenetic relationships." Mast and Thiele's work did not take up this challenge. It is to be hoped that Edwards et al. do so before making nomenclatural changes in Melaleuceae.

There has even been a move away from considering morphology useful in phylogenetic and taxonomic analyses, e.g., a paper by Scotland et al. (2003). The ultimate view is that of Tautz et al. (2003) who argued for taxonomy based solely on DNA, discussed with counter arguments by Lipscomb et al. (2003). A similar divergence of views in zoology was discussed by Mooi and Gill (2010). Morphology still has an important role in biological classification, but who knows where future theory will lead?

The Role of Australian Herbaria in Evaluating Nomenclatural Changes for the Australian Plant Census

The Australian Plant Census (APC) is a database of the accepted scientific names for the Australian vascular flora, both native and introduced, and lists synonyms and misapplications for these names (see <http://www.chah.gov.au/apc/about-APC.html>). The APC will cover all published scientific plant names used in an Australian context in the taxonomic literature, but excludes taxa (including cultivars) known only from cultivation in Australia. The APC "represents the considered opinion and nomenclatural research of [a convenor], 16 members of the APC Working Group, researchers that the Working Group consult in each herbarium, 8 Heads of Herbaria, 4 APC data entry people and user groups such as ABRS [Australian Biological Resources Study]" (Orchard, 2006: 30).

Discussing alternative taxonomies in relation to Australia's Virtual Herbarium (AVH), Entwisle and Weston (2005: 1) wrote that "for day-to-day business and pleasure, we [Australian taxonomists] must deliver 'what the customer wants.'" The same guidelines were to be used for the APC:

Guideline 1: Where possible, named taxa should be monophyletic based on current reliable evidence. This is qualified by Entwisle and Weston (2005: 2): ". . . there are times when we need to accept higher taxa [above species] that are not monophyletic, at least in the short term [earlier defined as 'e.g. 10 years'] . . . [such as] when different lines of evidence (especially molecular *v.* morphological) are in conflict." I believe that "monophyletic" is used here in its narrow sense, i.e., = holophyletic. As discussed above, paraphyletic taxa should be accepted, especially where supported by the morphology. In this case we have a monophyletic taxon (*Dryandra*) and a paraphyletic but easily defined one (*Banksia* sensu George, 1981, 1999a). Regardless of this, I suggest that this is a case where there is conflict between molecular and morphological evidence, and that acceptance of the transfer within a few months of publication was too rapid.

Guideline 2: Minimize taxonomic change (across Australia as a primary focus). In their discussion, Entwisle and Weston (2005: 2) say that "accepting stability . . . should result in both information gain and minimisation of nomenclatural confusion." The transfer of *Dryandra*, with 95 species and 41 infraspecific taxa arranged in a recently published infrageneric classification (George, 1996; Cavanagh & Pieroni, 2006), to *Banksia* and there placed in a single series, has done just the opposite—established some 135 new combinations and lost all the subdivision within *Dryandra*.

Guideline 3: Change is more acceptable in groups that are not "charismatic," not economically important, or do not have a substantial "interest group." Dryandras occur naturally only in Western Australia, where they are both charismatic and economically important. Further, there are significant "interest groups" for *Dryandra* and *Banksia* (Australian Native Plants Society Study Groups).

Guideline 4: The preferred "name" should be as scientifically defensible as possible, but its acceptance does not imply that it is necessarily the "best name" on scientific and/or social grounds. The scientific part of this guideline seems self-contradictory: perhaps it should be "as scientifically defensible as possible" yet not necessarily the "best name on scientific . . . grounds." I argue that *Banksia* s.l., as the current preferred name for the APC, is neither well justified scientifically nor the better name on social grounds.

Guideline 5: Avoid epithets already in use in possible congeners. This is not relevant to the current matter.

Guideline 6: The preferred name is that used in most states and territories ("majority rules"). The decision should still be based on good science. In 2004, the Council of Heads of Australian Herbaria established a committee to "make judgements on any contentious conflicts" (Entwisle & Weston, 2005: 4). This is discussed further below.

Mast and Thiele (2007) referred to these guidelines but cited only numbers 1 and 2. They agreed with the criteria but ignored Entwisle and Weston's qualifiers (2005). They wrote: "monophyly is sensible at ranks higher than species, given that this restriction makes taxa more predictive of character-state distributions, and provides a clearer reflection of phylogenetic relationships than when non-monophyletic taxa are permitted" (p. 64). Why does monophyly (holophyly) make taxa "more predictive of character-state distributions" and provide "a clearer reflection of phylogenetic relationships than when non-monophyletic taxa are permitted" (p. 64)? It's easy to say that X is (closely) related to Y, and a lot easier to discuss genera X and Y than genus X subgenus X and genus X subgenus Y. Also, the larger a genus, the larger and more complex the range of characters and states, hence the more difficult it may be to be predictive and see relationships within it. Biological names are about communication.

In the preface (pp. i, ii) to the special issue of *Australian Systematic Botany* (Barrett et al., 2005) in which these guidelines and the article by Mast et al. (2005) were published, the editors (Barrett, Hopper, and Farrer, though attributed to only Barrett in the Contents) commented several times on the need for caution before accepting some generic changes. They wrote: "we can best serve the end-users of our classifications by maintaining '*status quo*' until we are confident that our data are sufficient to provide a stable classification" (p. i); and later "In the case of *Banksia/Dryandra*, it is easy to recognize two 'genera' on morphological grounds and many will prefer to do so, even though this renders *Banksia* paraphyletic. The classification as two genera is considered by some to be more practical, even if it does not reflect true genetic relationships in the group concerned" (p. i). As stated above, even Mast and Thiele (2007) considered their results to be preliminary, stating that their new classification "is the least disruptive option *at present* [my italics] (p. 64)."

Discussing changes in the nomenclature of orchids, Barker and Bates (2008: 101) wrote that "Herbaria, among other institutions, tend to adopt a conservative approach in the adoption of new names, preferring to wait until there has been sufficient testing of new concepts and hence greater stability and acceptance of these names . . . Rushing in and adopting name changes as they occur can lead to a later reversal of a decision and an unnecessary confusion of names." Strict adherence to holophyletic taxa is also counterproductive for the users of plant names—see, for example, Brickell et al. (2008). When well-known, morphologically distinguishable, and identifiable genera are merged, it causes confusion, hinders communication, and can lead to loss or obscuring of information for those users. Examples (summarized by Brickell et al., 2008) include placing the Cactaceae in Portulacaceae, *Rafflesia* in Euphorbiaceae, and placing six well-known genera in *Primula*.

Farjon (2007: 641) wrote: "Taxonomy should be a science that earns its respect from users as well as fellow scientists. If it insists on strict adherence to a spurious dictum, more appropriate to a religious sect than to science, it will lose that respect."

Grant (2003: 1268) also wrote about the roles of taxonomic and cladistic products in society:

> There is a long-standing social need for similarity/difference classifications. This is why they have existed since prehistoric times. . . . In modern societies, taxonomic classifications are used for identification and information retrieval by the general public, by people in technological fields, and in biological research (including cladistics).
>
> Cladistics has no such general constituency. Phylogeny is of only secondary interest to most people including most kinds of biologists. Who needs a crocodile-bird clade when we already have a bird taxon and a crocodile taxon?

All these guidelines and considerations advise caution when deciding whether to adopt taxonomic and nomenclatural changes for the APC, especially of large groups. Yet the APC committee has ignored them in the case of *Banksia* and *Dryandra*. As far as I am aware, there has been no published report on how the Group reached its decision to accept *Banksia* s.l. or whether there was any discussion. Members agreed in response to a request by e-mail (B. Lepschi, CANB, in correspondence). A letter on the subject to the editor of

the Friends of the Australian National Botanic Gardens Newsletter no. 61 (<http://friendsanbg.org.au/newweb_drupal/sites/default/files/pdf/newsmar09.pdf>), reprinted in the *Dryandra* Study Group Newsletter no. 57, states just that the "Australian Plant Census ... represents the consensus view of Australian state and territory herbaria, as ratified by the Council of Heads of Australasian Herbaria" (Lepschi et al., 2009).

The website for the APC (<http://www.anbg.gov.au/cgi-bin/apclist>; accessed 22 April 2010) tells us only that *Dryandra* is a "synonym of Banksia L. f. nom. cons." However, many people with a working knowledge of these plants have rejected the merger. For example, the Banksia and Dryandra Study Groups have considered the merger and rejected it. The Wildflower Society of Western Australia continues to use *Dryandra*. Notably, the Botanic Gardens of Adelaide listed *Dryandra* in their 2010 catalogue of plants being grown there, despite the Gardens' own herbarium accepting the merger three years earlier. Also, in a paper coauthored by Mast (Sauquet et al., 2009), *Banksia* and *Dryandra* were considered as genera except for their final analysis: "For practical reasons [not explained], we do not follow this nomenclatural change [the merger of the two genera] here but we have included several species of *Banksia* as terminal taxa in our dataset and only consider Banksiinae (the monophyletic group of all species of *Banksia* and *Dryandra*) as a terminal taxon in all diversification rate analyses." In the paper, tables S5 (species numbers) and S6 (voucher information and GenBank accessions) list *Banksia* and *Dryandra* separately. He et al. (2011) retained *Banksia* s. str. Thus, we are left with the situation of Australian herbaria using one nomenclature and the "customers" another.

Conclusion

The molecular analysis of *Dryandra*, though limited in scope, has supported its close relationship to *Banksia*, but the decision to merge two genera that are iconic in the flora of southwestern Australia is based upon insufficient research and a concept that genera cannot be paraphyletic. Phylogenetic analysis can provide a useful hypothesis, but the results should be used cautiously, and not used to make premature changes in classification that may later be overturned. As Hörandl and Stuessy (2010: 1650, 1651) wrote, "Paraphyletic groups routinely originate as part of the evolutionary process, and they, therefore, have an important role to play in classification. ... Paraphyletic and holophyletic status should be used as features of taxa, describing their evolutionary status, not as criteria for their acceptance in classification." In a way it seems that, with strict cladistics, we have come full circle from the artificial system of taxonomy of Linnaeus to the artificial system of cladistics. Significantly, while the research discussed here may make *Banksia* paraphyletic with respect to *Dryandra*, it has not demonstrated conclusively that *Banksia* and *Dryandra* together are holophyletic. Those who know *Dryandra* and *Banksia* well accept the close relationship and have no difficulty distinguishing them on morphological grounds. Under the International Code of Nomenclature for algae, fungi, and plants (McNeill et al., 2012) there is no requirement to accept this merger, and many users of plant names have continued to use *Dryandra* and *Banksia*. Australian herbaria should follow their own guidelines and do likewise.

Two apomorphic states are clear cut in distinguishing *Dryandra* from *Banksia* s. str.—the receptacle is flat or only slightly convex or concave, and the common and floral bracts are openly arranged. In *Banksia* the receptacle is ovoid to cylindrical, and the common and floral bracts are tightly packed.

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PARAPHYLY AND ENDEMIC GENERA OF OCEANIC ISLANDS: IMPLICATIONS FOR CONSERVATION[1]

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ABSTRACT

Genera of flowering plants that are endemic to oceanic islands are often of great biological interest. These groups represent adaptive complexes that confer distinction to the islands or archipelagos in which they are found, and this often results in a focus on their conservation. In recent decades, numerous molecular phylogenetic (and other evolutionary) studies have been done on island genera, hence providing much valuable new information on relationships and evolution of island groups. Genera restricted to oceanic islands derive evolutionarily from parental stocks usually in continental regions. These parental genera are often themselves evolutionarily successful, being particularly adept at dispersal, adaptation, and speciation. These immigrants to isolated oceanic islands derive from common ancestors of large and diverse parents or directly from within the lineages themselves. If in the latter case the island derivatives are treated at the generic level, then the parental genus becomes paraphyletic in a cladistic sense. In this circumstance there are three alternatives to classification of the island group: (1) treat both the island complex as a distinct holophyletic genus and the progenitor as a coordinate, but paraphyletic, genus; (2) submerge the island complex into the parental genus, perhaps at the subgeneric or sectional level, creating a larger holophyletic genus; or (3) divide the parental genus and island complex into a series of smaller genera in such a manner that all become holophyletic. A synthesis of recent investigations on 100 endemic island genera and relatives was completed in the Bonin Islands, Canary Islands, Galápagos Islands, Hawaiian Islands, Madeiran Islands, Robinson Crusoe Islands, and St. Helena. The results show that 64 genera are still accepted and remain uninvestigated or are seen as holophyletic in phylogenetic analyses. Seven have already been submerged based on non-cladistic results, and 29 are viewed as being nested within larger parental genera. Of this latter group, 15 of the genera are still being recognized at this time; six have been recommended as belonging within their parental genera; and eight have been formally transferred into the progenitor genera with combinations made. If further actions were to be taken based on strict holophyly, following the second alternative mentioned above, then these 29 genera would disappear as endemics in their islands or archipelagos. This would result in an overall average drop of 31.9% endemic genera in oceanic islands worldwide (based on the sample analyzed). With the third alternative, new generic concepts for the island and progenitor taxa would need to be worked out. Instead of recognizing genera on the basis of simple holophyly, genera should be based on cohesiveness, distinctness, and monophyly s.l. (i.e., including paraphyly and holophyly). A statistic is provided as a means for making these assessments quantitatively. The importance of unique and/or divergent character change for classification of island lineages is also stressed.

Key words: Adaptations, cohesiveness, distinctness, generic limits, holophyly, island evolution, monophyly, paraphyly, predictive quality.

Oceanic islands are fascinating natural laboratories for the study of evolution and biogeography. Surrounded by water, these volcanic land masses offer opportunities for understanding dispersal, divergence, adaptive radiation, and speciation. Over millions of years in isolation from continental relatives, island populations diverge to such an extent morphologically that they are considered taxonomically distinct species. With even more geological time and more morphological divergence, endemic genera result. One of the attractions of island floras, in fact, has been the high levels of endemic species and genera that they contain. Most studies of processes of evolution in archipelagos

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focus on endemic taxa, because these are the systems that have evolved in situ. Furthermore, conservation priorities in oceanic islands often focus on investigating and preserving populations of these endemic taxa. They are, in effect, regarded as the biological treasures of these island ecosystems due to their morphological uniqueness.

New molecular phylogenetic (and other evolutionary) studies have now provided much new information on relationships among island taxa and their continental relatives. This has led to more insights on evolutionary relationships of endemic taxa. In a phylogenetic context, this has provided more precise assessment of holophyly and paraphyly (Hörandl & Stuessy, 2010) than was ever before possible. This applies not only to the island endemics, but also to the continental relatives, or progenitors.

Considerable discussion has prevailed over the merits of holophyly and paraphyly in biological classification. Many workers admit only holophyletic groups in classification on the grounds that this is the only way to have classification related precisely to phylogeny, and vice versa (Hennig, 1966; Ebach & Williams, 2004; Schmidt-Lebuhn, 2011; Wiley & Lieberman, 2011). Strict adherence to a branching topology as an evolutionary explanation does provide a clear basis for hierarchical classification, but it does not take evolutionary divergence within lineages into account. Workers who believe that this evolutionary information should also be incorporated into biological classification may elect to recognize both holophyletic and paraphyletic groups (Stuessy, 1997; Brummitt, 2002; Hörandl, 2007; Hörandl & Stuessy, 2010).

Application of strict holophyly is of particular importance at the generic level. Many studies over the past several decades, especially in view of new molecular data and phylogenetic analyses, have led to generic remodeling. If a genus is shown to be paraphyletic, the solutions have been either to lump together the units involved into one larger genus, or to split up the group into several (or even many) smaller holophyletic genera. In oceanic islands, the latter approach could lead to an increase in numbers of island endemic genera. The former approach, however, would see the submergence of formerly endemic island genera into larger continental genera.

Evidence to date suggests that there is a tendency for lumping island endemic genera into larger continental relatives. The island genera are derived by dispersal and divergence from continental progenitors. Because of the speed of the divergence, it might be that the island genera are genetically not so divergent from the continental relatives, but they are usually very divergent morphologically, hence their recognition at the generic level. As an example, in the Robinson Crusoe (Juan Fernández) Islands, recently the two largest endemic genera, *Robinsonia* and *Dendroseris* (Compositae) with seven and 11 endemic species, respectively, were transferred into the large *Senecio* (Pelser et al., 2007, 2010a, 2010b) and *Sonchus* (Mejías & Kim, 2012) genera.

It is appropriate, therefore, to examine the impact that application of concepts of holophyly, based primarily on molecular phylogenetic analyses, has already had, or might potentially have, on levels of generic endemism in oceanic archipelagos worldwide. Depending upon how broad the effects might be, these actions could have a substantial impact on world island conservation. Furthermore, as genetic endemism is a criterion for delimitation of floristic zones (e.g., Takhtajan, 1986), a reduction in endemic genera in oceanic islands might also have an impact on the classification of world vegetation.

Therefore, the objectives of this paper are to: (1) inventory the genera endemic to representative archipelagos of the world, viz., Bonin Islands (Japan), Canary Islands (Spain), Galápagos Islands (Ecuador), Hawaiian Islands (U.S.A.), Madeiran Islands (Portugal), Robinson Crusoe Islands (Chile), and St. Helena (United Kingdom); (2) survey the endemic genera in these archipelagos that have new molecular phylogenetic (or other recent evolutionary) studies; (3) determine the effect that adherence to strict holophyly will (or might) have on existing generic limits and, therefore, on potential conservation mandates; and (4) suggest a method other than strict holophyly for evaluation of generic limits as determined by predictive quality (defined by a combination of monophyly s.l., distinctness, and cohesiveness).

Genera Endemic to Oceanic Archipelagos

The first step in examining the status of endemic genera in oceanic islands of the world is to obtain an inventory of the taxa. We concentrate on angiosperms only, as these have similar patterns of biogeography and evolutionary divergence that contrast with these aspects in the ferns (e.g., Stuessy et al., 1990).

We have elected to examine seven different oceanic island systems that are representative of size, complexity, and geographical position, and that also have had at least some recent molecular phylogenetic attention. These are (with political affinities): Bonin Islands (Japan), Canary Islands (Spain), Galápagos Islands (Ecuador), Hawaiian Islands (U.S.A.), Madeiran Islands (Portugal), Robinson Crusoe (= Juan Fernández) Islands (Chile), and St. Helena (United Kingdom). Lists of endemic genera were developed by consulting major floristic

Table 1. Total number of endemic angiosperm genera and included species of selected oceanic islands or archipelagos.

Island/Archipelago	Number of endemic genera	Number of included species
Hawaiian Islands	34	344
Canary Islands	30	150
Robinson Crusoe Islands	12	31
St. Helena	10	15
Galápagos Islands	7	25
Madeiran Islands	5	9
Bonin Islands	2	3
Totals	**100**	**577**

treatments for these islands/archipelagos as follows: Bonin Islands (Ono & Kobayashi, 1985), Canary Islands (Hansen & Sunding, 1993), Galápagos Islands (Wiggins & Porter, 1971), Hawaiian Islands (Wagner et al., 1999), Madeiran Islands (Press & Short, 1994), Robinson Crusoe Islands (Marticorena et al., 1998; Danton et al., 2006), and St. Helena (Cronk, 2000).

A summary of genera endemic in these selected oceanic islands is given in Table 1. A total of 100 genera has been described and recognized in these seven islands/archipelagos. The Hawaiian Islands contain the most (34) genera and the small Bonin Islands the fewest (2). In general, the degree of generic divergence (and endemism) relates to the size of the island chain and its isolation from source areas. The number of included species in these endemic genera is 577, with 59% of these being in the Hawaiian Islands. Levels of specific diversity in islands relate directly to elevation and habitat heterogeneity (Stuessy et al., 2006). This large number of species is also of interest in that should the genera be remodeled due to holophyly (or other reasons), names of some of these endemics would perforce need to be changed, which would create some inconvenience with conservation agendas.

Recent Molecular Phylogenetic (or Other Evolutionary) Studies on Endemic Genera and Their Taxonomic Impact

In this molecular age, it is hardly surprising that a significant number of studies have focused on phylogenetic (and broader evolutionary) relationships within endemic island genera and among these genera and their presumptive continental relatives. Within the genera, the focus has obviously been on resolving affinities among the endemic species, particularly to assess specific monophyly s.l., evaluate character evolution, and infer modes of speciation. Among genera, the objective has been principally to determine biogeographic connections and resolve taxonomic issues at the generic level. This section of the paper examines these new investigations (summarized in Table 2) and reveals their implications for the taxonomic status of endemic genera in selected oceanic islands and archipelagos.

Bonin Islands

These islands, also known as the Osagawara Islands, are an archipelago of 20 small land masses located approximately 1000 km south of mainland Japan. They harbor a total flora of 460 taxa of vascular plants, of which 30% are endemic (Ono, 1998), including two endemic genera, *Dendrocacalia* and *Boninia* (Ito, 1998). *Dendrocacalia* (Compositae, Senecioneae), a dioecious tree consisting of only one species, *D. crepidifolia*, is restricted to Haha Island within the archipelago (Kato & Nagamasu, 1996). A recent molecular phylogenetic survey of tribe Senecioneae (Pelser et al., 2007) included *Dendrocacalia*, and it resolved sister to *Arrhenechthites novoguineensis*. However, due to conflicts between nuclear and plastic gene markers, as well as the consideration of the existence of many smaller genera in relation to the huge *Senecio*, much more study is needed before generic limits in this tribe can be finalized. In the meantime, *Dendrocacalia* remains independent and, hence, as an endemic genus in the Bonin Islands. It is treated as distinct in the recent Compositae book (Funk et al., 2009).

The other endemic genus described for the Bonin Islands has been *Boninia* (Rutaceae), described originally by Planchon (1872), and consisting of two species, *B. grisea* and *B. glabra*. Morphological studies on these species, which were treated within a recent revision of the related genera *Euodia* and *Melicope* (Hartley, 2001), have revealed that *B. glabra* is actually a taxonomic synonym of *M. quadrilocularis*. *Boninia grisea* remains as a good species, but based on morphology, it appears to fall more properly within the large *Melicope* as *M. grisea*. Harbaugh et al. (2009) have examined molecular phylogenetic relationships within *Melicope*, but no samples of the former *Boninia* were included. Evidence to date, therefore, suggests that *Boninia* should be included within *Melicope*, and the former disappears as an endemic genus in the Bonin Islands.

Canary Islands

The Canary Islands, belonging to Spain, and being a favorite holiday destination for Europeans, have received considerable molecular phylogenetic (and other evolutionary) attention. There are 30 endemic

Table 2. Genera of angiosperms considered previously to be endemic to representative oceanic islands/archipelagos of the world and recent molecular phylogenetic (or other evolutionary) studies.

Island group and endemic genus	Family	Number of species	References
Bonin Islands			
Boninia	Rutaceae	2	Hartley, 2001
Dendrocacalia	Compositae, Senecioneae	1	Pelser et al., 2007
Canary Islands			
Aeonium	Crassulaceae	38 (plus 1 in Africa and 1 in Madeira)	Mes et al., 1997; Mort et al., 2001, 2002
Aichryson	Crassulaceae	10	Mes et al., 1997; Mort et al., 2001, 2002
Allagopappus	Compositae, Inuleae	2	Andrus et al., 2004
Argyranthemum	Compositae, Anthemideae	24	Francisco-Ortega et al., 1995a, 1995b, 1997
Atalanthus (= *Taeckholmia*)	Compositae, Lactuceae	8	Whitton et al., 1995; Kim et al., 1996a, 1996b, 2007
Babcockia	Compositae, Lactuceae	1	Whitton et al., 1995; Kim et al., 1996a, 1996b, 2007
Benconia	Rosaceae	4	Helfgott et al., 2000
Bethencourtia	Compositae, Senecioneae	2	Pelser et al., 2007, 2010
Bystropogon	Lamiaceae	7	Trusty et al., 2004, 2005
Dendriopoterium	Rosaceae	2	Helfgott et al., 2000
Dicheranthus	Caryophyllaceae	1	Fior et al., 2006
Gesnouinia	Urticaceae	1	Monro, 2006; Hadiah et al., 2008
Gonospermum	Compositae, Anthemideae	4	Francisco-Ortega et al., 2001a
Greenovia	Crassulaceae	4	Mes et al., 1997; Mort et al. 2001, 2002; Kim et al., 2008
Heywoodiella	Compositae, Lactuceae	1	Lack, 1978
Ixanthus	Gentianaceae	1	Thiv et al., 1999
Kunkeliella	Santalaceae	4	Der & Nickrent, 2008
Lactucosonchus	Compositae, Lactuceae	1	Whitton et al., 1995; Kim et al., 1996a, 1996b, 2007
Lugoa	Compositae, Anthemideae	1	Francisco-Ortega et al., 2001a
Marcetella	Rosaceae	2	Helfgott et al., 2000
Monanthes	Crassulaceae	18	Mes et al., 1997; Mort et al., 2001, 2002
Neochamaelea	Rutaceae	1	Appelhans et al., 2010
Parolinia	Brassicaceae	2	—
Plocama	Rubiaceae	1	Backlund et al., 2007
Schizogyne	Compositae, Inuleae	2	Francisco-Ortega et al., 2001b
Spartocytisus	Leguminosae	2	Cubas et al., 2002; Pardo et al., 2004
Sventenia	Compositae, Lactuceae	1	Whitton et al., 1995; Kim et al., 1996a, 1996b, 2007
Tinguarra	Umbelliferae	2	Downie et al., 2000; Spalik et al., 2001
Todaroa	Umbelliferae	1	Downie et al., 2000; Spalik et al., 2001
Vieraea	Compositae, Inuleae	1	Francisco-Ortega et al., 2001b; Andrus et al., 2004
Galápagos Islands			
Brachycereus	Cactaceae	1	Lendel et al., 2006
Darwiniothamnus	Compositae, Astereae	3	Andrus et al., 2009
Jasminocereus	Cactaceae	1	Applequist & Wallace, 2002; Lendel et al., 2006
Lecocarpus	Compositae, Millerieae	3	Blöch et al., 2009; Stuessy et al., 2011
Scalesia	Compositae, Heliantheae	15	Schilling et al., 1994
Sicyocaulis	Curcurbitaceae	1	Sebastian et al., 2010
Trigonopterum	Compositae, Ecliptinae	1	Panero et al., 1999; Wagner & Robinson, 2001
Hawaiian Islands			
Alsinodendron	Caryophyllaceae	4	Wagner et al., 2005
Argyroxiphium	Compositae, Madieae	5	Baldwin, 2003
Bobea	Rubiaceae, Guettardeae	4	Achille et al., 2006; Bremer & Eriksson, 2009
Brighamia	Campanulaceae	2	Givnish et al., 1995, 2009; Antonelli, 2008
Broussaisia	Hydrangeaceae	1	Hufford et al., 2001; Xiang et al., 2011

Table 2. Continued.

Island group and endemic genus	Family	Number of species	References
Clermontia	Campanulaceae	22	Givnish et al., 1995, 2009; Antonelli, 2008
Cyanea	Campanulaceae	52	Givnish et al., 1995, 2009; Antonelli, 2008
Delissea	Campanulaceae	9	Givnish et al., 1995, 2009; Antonelli, 2008
Dissochondrus	Gramineae	1	—
Dubautia	Compositae, Madieae	21	Baldwin, 2003
Haplostachys	Labiatae	5	Lindqvist & Albert, 2002; Lindqvist et al., 2003; Scheen et al., 2010
Hesperomannia	Compositae, Vernonieae	3	Kim et al., 1998; Keeley et al., 2007
Hibiscadelphus	Malvaceae	7	—
Hillebrandia	Begoniaceae	1	Clement et al., 2004; Forrest et al., 2005
Isodendrion	Violaceae	4	Tokuoka, 2008
Kokia	Malvaceae	4	Seelanan et al., 1997; Pfeil et al., 2002
Labordia	Loganiaceae	15	Bremer & Struwe, 1992; Motley & Carr, 1998; Backlund et al., 2000
Lipochaeta	Compositae, Heliantheae	6	Rabakonandrianina, 1980; Rabakonandrianina & Carr, 1981; Panero et al., 1999; Wagner & Robinson, 2001
Munroidendron	Araliaceae	1	Plunkett et al., 2001, 2004a, 2004b; Lowry & Plunkett, 2010; Plunkett & Lowry, 2010
Neraudia	Urticaceae	5	—
Nothocestrum	Solanaceae	4	Olmstead et al., 2008
Nototrichium	Amaranthaceae	3	Kadereit et al., 2003; Müller & Borsch, 2005; Ogundipe & Chase, 2009
Pelea	Rutaceae	48 (plus 2 in Marquesas)	Hartley & Stone, 1989; Hartley, 2001
Phyllostegia	Labiatae	32 (plus 1 in Tahiti)	Lindqvist & Albert, 2002; Lindqvist et al., 2003; Scheen et al., 2010
Platydesma	Rutaceae	4	Hartley, 2001; Harbaugh et al., 2009
Pteralyxia	Apocynaceae	2	Livschultz et al., 2007
Remya	Compositae, Astereae	3	Karaman-Castro & Urbatsch, 2009
Rollandia	Campanulaceae	8	Lammers et al., 1993; Givnish et al., 1995
Schiedea	Caryophyllaceae	34	Wagner et al., 1995, 1999, 2005; Soltis et al., 1996; Weller et al., 2001; Kapralov & Filatov, 2006; Sakai et al., 2006; Willyard et al., 2011
Stenogyne	Labiatae	20	Lindqvist & Albert, 2002; Lindqvist et al., 2003; Scheen et al., 2010
Tetraplasandra	Araliaceae	7	Costello & Motley, 2001, 2007
Touchardia	Urticaceae	1	Monro, 2006
Trematolobelia	Campanulaceae	4	Givnish et al., 2009; Lammers, 2009
Wilkesia	Compositae, Madieae	2	Baldwin, 2003
Madeiran Islands			
Chamaemeles	Rosaceae	1	—
Monizia	Umbelliferae	1	Spalik & Downie, 2007
Musschia	Campanulaceae	2	Roquet et al., 2008
Parafestuca	Gramineae	1	Quintanar et al., 2006
Sinapidendron	Cruciferae	4	Warwick & Black, 1993; Warwick & Sauder, 2005
Robinson Crusoe Islands			
Centaurodendron	Compositae, Cardueae	2	Susanna et al., 2011
Cuminia	Labiatae	2	Trusty et al., 2004; Bräuchler et al., 2010
Dendroseris	Compositae, Lactuceae	11	Kim et al., 2007; Mejías & Kim, in press
Juania	Palmae	1	—
Lactoris	Lactoridaceae	1	Soltis et al., 1997, 2011; Gonzalez & Rudall, 2001; Wanke et al., 2006
×*Margyracaena*	Rosaceae	1	Crawford et al., 1993
Megalachne	Gramineae	2	—

Table 2. Continued.

Island group and endemic genus	Family	Number of species	References
Nothomyrcia	Myrtaceae	1	Murilla-Aldana & Ruiz, 2011; Murillo-A. et al., 2012
Rhetinodendron	Compositae, Senecioneae	1	Pacheco et al., 1985; Sanders et al., 1987; Sang et al., 1995; Pelser et al., 2007, 2010b
Robinsonia	Compositae, Senecioneae	7	Pelser et al., 2007, 2010b
Selkirkia	Boraginaceae	1	—
Yunquea	Compositae, Cardueae	1	—
St. Helena			
Commidendrum	Compositae, Astereae	4	Eastwood et al., 2004
Lachanodes	Compositae, Senecioneae	1	Pelser et al., 2010a
Melanodendron	Compositae, Astereae	1	Eastwood et al., 2004
Mellissia	Solanaceae	1	Olmstead et al., 2008
Nesiota	Rhamnaceae	1	—
Nesohedyotis	Rubiaceae	1	Bremer & Eriksson, 2009; Groeninckx et al., 2009
Petrobium	Compositae, Coreopsideae	1	Ryding & Bremer, 1992; Kimball & Crawford, 2004
Pladaroxylon	Compositae, Senecioneae	1	Pelser et al., 2010a
Trimeris	Campanulaceae	1	Lammers, 2011
Trochetiopsis	Sterculiaceae	3	—

genera, including *Aeonium* (Crassulaceae) being mostly endemic (with one species also in Africa). Several genera are more properly endemic to Macaronesia, which includes the Madeiran Islands, Salvage Islands, and also the Azores.

One endemic genus, *Parolinia* (Brassicaceae), with two recognized species, *P. glabriuscula* and *P. platypetala*, has not yet been investigated in a modern phylogenetic context. Patterns of isozymic genetic variation in both species, however, have been examined (Fernandez-Palacios et al., 2004). Morphological studies have allied *Parolinia* variously with *Diceratella*, *Leiospora*, *Maresia*, *Morettia*, etc. (Al-Shehbaz et al., 2006).

Two genera have been removed from the endemic ranks based on non-cladistic (or non-molecular) investigations. *Heywoodiella* (Compositae, Lactuceae) was described by Sventenius and Bramwell (1971) as endemic to the one island, Tenerife, but earlier comparative morphological and cytological investigations (Lack, 1978) have shown it to be a species of *Hypochaeris*, which has ca. 15 species in the Mediterranean region plus 41 in South America (E. Urtubey et al., in prep.). *Neochamaelea* (Rutaceae) is another monospecific genus, in this case related to *Cneorum*. Recent comparative morphological studies (Appelhans et al., 2010) have led to synonymizing *Neochamaelea* into *Cneorum*.

Two endemic species of *Senecio*, *S. hermosae* and *S. palmensis*, were earlier placed in a separate endemic genus, *Bethencourtia* (Compositae, Senecioneae), by Choisy (in Buch, 1825). These were subsequently brought back to *Senecio* but kept as a distinct section by de Candolle (1838). Nordenstam (2006) again elevated the section to generic level, plus adding a new species, but he coined the name *Canariothamnus*, because he believed *Bethencourtia* to be invalidly published (apparently because it was not accepted by Buch in the original work). A closer nomenclatural examination (Nordenstam in Greuter & Raab-Straube, 2006), however, revealed that *Bethencourtia* was, indeed, validly published because it was accepted by the describing author, Choisy (1825), hence relegating the recent *Canariothamnus* to synonymy. Molecular phylogenetic studies (ITS) of *Senecio* (Pelser et al., 2007) have shown *Bethencourtia* to form a small holophyletic group, which ties closest with species of *Jacobaea* (from Eurasia) and then with sub-Saharan African species of *Senecio*. With ITS/ETS combined data (Pelser et al., 2010a), *B. palmensis*, *J. vulgaris*, and *S. lineatus* form a well-supported clade (97% bootstrap support [BS]). With plastid data (Pelser et al., 2010a: 863), *Bethencourtia* is given as "sister to *Emilia*" (77% BS; table 3). Due to the many species of *Senecio* and uncertainty regarding how to properly circumscribe genera in this massive complex, no further taxonomic action has been taken at this point. At the present time, therefore, *Bethencourtia* remains an endemic genus in the Canary Islands.

Many of the recent molecular phylogenetic studies on endemic genera have confirmed holophyly for

several of them; therefore, they retain endemic status. *Gesnouinia* (Urticaceae) is a monospecific genus (*G. arborea*) examined in context of molecular phylogenetic studies on the entire family using *rbcL* and *trnL-F* chloroplast markers (Monro, 2006; Hadiah et al., 2008). The genus appears as one of the basal lineages, tying with good support (100% BS) to *Parietaria pensylvanica*, and these both nested in a polytomy with three other genera (Monro, 2006). *Bystropogon* (Lamiaceae) consists of seven species, with two restricted to the Madeiran Islands. Recent molecular phylogenetic studies with ITS, 5.8S, *trnL*, and *trnL-trnF* (Trusty et al., 2004, 2005) reveal the genus to be holophyletic (with moderate BS). *Dicheranthus* (Caryophyllaceae) is a monospecific (*D. plocamoides*) genus of tribe Paronychieae (Bittrich, 1993). Molecular phylogenetic investigations with *matK* and ITS (Fior et al., 2006) reveal the genus to be sister to *Pteranthus dichotomus*. *Ixanthus* (Gentianaceae) is a monospecific endemic genus of uncertain generic affinities generally within subtribe Gentianinae (Yuan & Küpfer, 1995). Molecular phylogenetic studies by Thiv et al. (1999), using ITS and *matK*, however, show *I. viscosus* to be sister to three species of *Blackstonia* (total four species from Europe and the Mediterranean region), and within subtribes Erythraeinae or Chironiinae.

Three of the endemic holophyletic genera are in Compositae. One of the largest genera in the Canary Islands is *Argyranthemum* (Anthemideae) with 24 species endemic to Macaronesia. Twenty species are endemic to the Canary Islands proper, with four others found in the Madeiran Islands, Salvage Islands, and the Azores. ITS (Francisco-Ortega et al., 1997) and chloroplast DNA (cpDNA) studies (Francisco-Ortega et al., 1995a, 1995b, 1997) have confirmed holophyly of this genus, which ties to a clade that includes species of *Heteranthemis*, *Ismelia*, and *Chrysanthemum*. *Vieraea* is a monospecific (*V. laevigata*) endemic genus belonging to tribe Inuleae and related to *Pulicaria*. Molecular phylogenetic studies using ITS (Francisco-Ortega et al., 2001b; Andrus et al., 2004) have examined these and related genera and show that the genus is sister (74% BS) to *Perralderia coronopifolia*, representing a genus of three species of northwestern Africa (Mabberley, 2008). On morphological grounds, *Vieraea* appears closer to *Pulicaria vieraeoides* (Andrus et al., 2004). *Allagopappus* is a bispecific endemic genus also of tribe Inuleae. In the same study by Andrus et al. (2004) using ITS sequences, *Allagopappus* resolves in a distinct basalmost Macaronesian lineage and, therefore, is still recognized as endemic in the archipelago.

Other genera have been investigated with new methods and represent possible taxonomic changes, but the authors involved have not yet taken formal actions. *Kunkeliella* (Santalaceae) is a genus of four species that is close to *Thesium* and related genera (Stearn, 1972). The new molecular phylogenetic studies (Der & Nickrent, 2008), however, show *K. subsucculenta* to be sister to *Thesidium* (*T. fragile*) and these two sister to two analyzed species of *Thesium* in the "Thesium Clade." *Kunkeliella*, therefore, at this time remains a good endemic genus, although Stearn (1972) suggested that as another alternative, it might be regarded as a new section of *Thesium*.

Within the archipelago is a conspicuous woody Compositae alliance of 11 species centered around *Sonchus* that includes the endemic genera *Babcockia*, *Lactucosonchus*, *Sventenia*, and *Taeckholmia*, the first three being monospecific and the last with eight species. *Taeckholmia* has now been combined into *Atalanthus* (Hansen & Sunding, 1993: 6), because the former name was discovered to be illegitimate. Whitton et al. (1995), using cpDNA restriction site data, began to obtain a clearer view of relationships among genera within the tribe, and Kim et al. (1999) followed with cpDNA (*psbA-trnH* intergenic spacer) sequences that gave somewhat better resolution. ITS data (Kim et al., 1996a, 1996b), however, have provided even more resolution. The four genera in question were accepted as distinct by Bremer (1993, 1994), and they were also listed in Kilian et al. (2009) but with the indication that generic limits relating to *Sonchus* need to be reconsidered. These same authors, however, in an appendix to their work (p. 381), listed all these endemic genera as synonyms under *Sonchus* but without combinations for included species. Kim et al. (2007), using ITS and *matK* sequences, show clearly that *Babcockia*, *Lactucosonchus*, and *Sventenia* fall within *Sonchus*, and that it was their intention to eventually make such transfers, the genera being reduced to subgenera. Such transfers have apparently not yet been completed, however.

The subtribe Gonosperminae of Compositae consists of three genera, two of which are endemic to the Canary Islands: *Gonospermum* (four species) and *Lugoa* (one species). All these species have been examined by ITS markers (Francisco-Ortega et al., 2001a) in comparison to related genera of tribe Anthemideae, and the results show that these two genera form a holophyletic group, although not highly supported. *Lugoa* falls within *Gonospermum*, and the former, therefore, is suggested to be an indistinct genus, but the formal combinations have not yet been

made. *Gonospermum* is holophyletic if *Lugoa* is combined into it.

Crassulaceae have long attracted attention for the many morphologically interesting endemic species in the Canary Islands, as well as in all of Macaronesia. The endemic (or nearly so) genera include *Aeonium* (38 species, plus one from Madeira and another from eastern Africa; Liu, 1989), *Aichryson* (10 species, with three species confined to the Madeiran Islands, and one also occurring in the Azores), *Greenovia* (four species), and *Monanthes* (18 species). That this forms a large holophyletic assemblage has been determined earlier by Mort et al. (2001). The relationships among the genera, however, have now also been examined (Mort et al., 2002) by ITS and several cpDNA regions (*matK*, and *trnL-trnF* and *psbA-trnH* spacers). The results show *Aichryson* to be holophyletic and sister to the other genera. *Monanthes* is nearly holophyletic, with *M. icterica* slightly more basal in the cladogram. This species was earlier shown to be somewhat divergent with ITS as well (Mes et al., 1997). *Aeonium* is solidly in one clade, but it is considered paraphyletic, because *Greenovia* (itself holophyletic) is nested inside and sister to *A. viscatum* and then next allied to *A. saundersii*. Mort et al. (2002) suggest including *Greenovia* into *Aeonium*, but no formal transfers have yet been made. Kim et al. (2008) also refer to *Aeonium* in the sense of *Greenovia* being included within it.

Spartocytisus (Leguminosae) with two species is an endemic genus related to *Cytisus* and allied genera. Molecular phylogenetic studies (Cubas et al., 2002) using ITS and *trnL-trnF* spacer show *S. supranubius* to be sister to a large clade with species of *Cytisus*, but including also a smaller holophyletic *Calicotome* and *Chamaecytisus proliferus*. Outside the clade fall other genera and five other species of *Cytisus*. The authors suggest that *Spartocytisus* might be "better considered as a separate section within *Cytisus* (i.e., sect. *Oreosparton*)" (Cubas et al., 2002: 239). Relationships of *Spartocytisus* were again examined by a study with the same markers but focusing on *Genista* and allied genera (Pardo et al., 2004); no change in relationships for *Spartocytisus* was observed.

Several molecular phylogenetic studies have resulted in new insights and taxonomic changes on relationships for endemic genera in the Canary Islands. *Plocama* (Rubiaceae) is an endemic monospecific genus (*P. pendula*) with morphological affinities with the genera *Crocyllis*, *Kellogia*, *Neogaillonia*, and *Putoria* in "Group 1" of tribe Paederieae (Puff, 1982). Molecular phylogenetic studies (Backlund et al., 2007) on genera of Paederieae using cp *rbcL*, *rps16* intron, and *trnT-F* show *Plocama* to form a small clade with *Crocyllis* and species of *Gaillonia* (*G. yemenensis* and *G. tinctoria*). The authors point to the importance of a larger clade, however, that includes also the genera *Putoria*, *Pterogaillonia*, *Pseudogaillonia*, *Aitchisonia*, *Jaubertia*, and *Choulettia*, the group being accorded tribal recognition as Putorieae. All these genera, however, have now been combined into one genus, as this basal node has high BS support (100%). The authors also stress that diagnostic morphological features to characterize these genera have been difficult to find. As *Plocama* is the earliest generic name, the genus has now been expanded from one to 34 species, and it ceases being a Canarian endemic.

Tinguarra (Umbelliferae) with two endemic species is confined to the Canary Islands (*T. sicula* from Europe was earlier submerged into *Athamanta* [Tutin, 1968], but this has not been entirely accepted [e.g., Knees, 1996]). Downie et al. (2000), using ITS1 and 2 sequences, showed close affinities of *Tinguarra* with *Athamanta*, a genus of about five Mediterranean species (Mabberley, 2008), suggesting that the two might be treated as a single genus (along with *Todaroa*). Combining ITS data with morphological and anatomical characters, Spalik et al. (2001) clearly showed *Tinguarra* to be sister to *Athamanta* and made formal transfers of *T. cerviariaefolia* and *T. montana* into *Athamanta* as section *Tinguarra*. The monospecific *Todaroa* (Umbelliferae) remains isolated in analysis with ITS plus morphological and anatomical data (Spalik et al., 2001), and hence still remains an endemic genus in the archipelago.

Finally, in a few instances the new molecular data and phylogenetic analyses have simply not yet been able to revolve relationships of some of the endemic genera, and more work will obviously be needed in the future. *Bencomia*, *Dendriopoterium*, and *Marcetella* are endemic genera of Rosaceae in Macaronesia, the former two genera including four and two species, respectively, from the Canary Islands. *Marcetella* has one species endemic to the Canary Islands (*M. moquiniana*) and one to Madeira and the Azores (*M. maderensis*). Molecular phylogenetic studies (Helfgott et al., 2000) based on ITS show this *Bencomia* alliance to be holophyletic and well supported (100% BS). *Dendriopoterium* and *Marcetella* were at one time (Nordberg, 1966) placed as sections together within *Sanguisorba* as subgenus *Dendriopoterium*, but *Bencomia* was left as a distinct genus. The ITS data poorly resolve the *Bencomia* alliance and, therefore, at this time these genera remain endemic to the islands. The data do show, however, that none of the genera belong in *Sanguisorba*.

Schizogyne (Compositae, Inuleae), with two species, is endemic to the Canary Islands (*S. sericea* being also known in the Salvage Islands; Hansen & Sunding, 1993), along with *Allagopappus* and *Vieraea*. Molecular phylogenetic studies using ITS (Francisco-Ortega et al., 2001b) reveal that *Schizogyne* is sister to three species of *Pulicaria*, which itself appears to be biphyletic, and together with the monospecific *Limbarda crithmoides* from Europe all form a good clade. No taxonomic actions have yet been implemented from these results, however, and *Schizogyne*, therefore, remains an endemic genus at this time.

Galápagos Islands

These well-known islands contain 309 genera of angiosperms (Wiggins & Porter, 1971), of which only seven are regarded as endemic (Table 1). In general, there have been few molecular phylogenetic investigations in the Galápagos Islands, perhaps due to restrictions on collecting activities in the archipelago. At present, all of the recognized endemic genera remain undisturbed with regard to taxonomic status. This includes genera documented as sister groups to other genera, as well as those nested within larger continental genera.

There are two endemic genera of cacti, *Brachycereus* and *Jasminocereus*, each with only one species (but the latter with three recognized varieties). These two genera apparently are closely related to each other and have affinities to *Armatocereus* from coastal Ecuador and Peru (Anderson, 2001, from a statement based on DNA sequences communicated by R. Wallace). Barthlott and Hunt (1993), however, placed *Jasminocereus* in tribe Browningeae and *Brachycereus* in Trochocereae. Some molecular sequence studies have been published dealing with Cactaceae (e.g., Nyffeler, 2002; Arias et al., 2005), but neither endemic genus from the Galápagos was sampled for these investigations. Applequist and Wallace (2002), however, did include *Jasminocereus* using data on deletions in the intergenic spacer *trnT-trnL* chloroplast region, but the tribal placement of this endemic genus is not well indicated. Lendel et al. (2006), in an online abstract, reported that based on cp *trnK* intron (including *matK*) and *rps16* intron, *Brachycereus* and *Jasminocereus* form a strongly supported group sister to *Armatocereus*. For the time being, at least, these two species of cacti remain as endemic genera in the islands.

Scalesia (Compositae, Heliantheae), the largest endemic plant genus in the islands with approximately 15 species, appears to be a monophyletic assemblage based on morphological and cytological data (Eliasson, 1974). Based on cpDNA restriction site analyses (Schilling et al., 1994), *Scalesia* relates to *Pappobolus* on the South American continent. Only one species of *Scalesia*, the abundant *S. pedunculata*, however, was sampled, and the authors admit that the conclusions are somewhat tenuous.

Four genera, *Darwiniothamnus*, *Lecocarpus*, *Sicyocaulis*, and *Trigonopterum*, have been shown from molecular phylogenetic studies to be nested within larger parental genera. Despite these perspectives, however, the genera have not been submerged at this time (with or without formal combinations). *Darwiniothamnus* (Compositae, Astereae) is a shrubby endemic genus of three species. Molecular phylogenetic studies using ITS (Andrus et al., 2009) have demonstrated it to be biphyletic, with *D. lancifolius* and *D. tenuifolius* relating to *Erigeron bellidiastroides* of the Caribbean region, and with *D. alternifolius* tying to two Chilean species of *Erigeron*. The authors do not state that *Darwiniothamnus* should be submerged into *Erigeron*, but as a biphyletic entity deriving from two different clades of this latter genus, there could hardly be any other alternative if holophyly were applied as a classificatory perspective for this group. At the present time, however, *Darwiniothamnus* still survives as an endemic genus in the archipelago.

Lecocarpus, another woody endemic genus of Compositae (Millerieae) in the Galápagos Islands, consists of three morphologically distinct species (Adsersen, 1980). Molecular phylogenetic studies using ITS and *matK* have been completed on this genus (Blöch et al., 2009; Stuessy et al., 2011) along with the larger genus *Melampodium* (40 species) and another small close generic relative, *Acanthospermum* (six species). The results clearly show *Lecocarpus* (and *Acanthospermum*) to be nested within the large and morphologically diverse *Melampodium*. This latter genus is distributed throughout Mexico and Central America, whereas *Acanthospermum* is more concentrated in Central America and northern South America (Blake, 1922). It would not be surprising that *Lecocarpus* might have dispersed from ancestral populations of *Acanthospermum* and diverged rapidly in the oceanic island environment. Species of *Lecocarpus* are woody shrubs with conspicuous white-flowering heads, fruits with wide hoods or long awns, and highly distinctive dissected leaves, quite unlike features known in *Melampodium* or *Acanthospermum*. Because of this striking divergence, and because of the biogeographic hypothesis of origin of *Lecocarpus* from out of *Acanthospermum* (or its immediate ancestor) into the islands, Stuessy et al.

(2011) elected to retain *Lecocarpus* as a separate and, therefore, endemic genus.

The endemic *Sicyocaulis* (Cucurbitaceae), with the single species *S. pentagonus*, has been shown recently from molecular phylogenetic studies involving several nuclear and chloroplast DNA sequences to be nested within the larger genus *Sicyos* (Sebastian et al., 2010). Despite this clear relationship, no suggestion that *Sicyocaulis* should be submerged into *Sicyos*, however, was proposed by the authors. A broad survey of the family has also been completed (Schaefer et al., 2009; Schaefer & Renner, 2011), but *Sicyocaulis* was not included in the samples reported for these analyses.

The endemic *Trigonopterum* (Compositae, Ecliptinae; formerly *Macraea*, nom. illeg.; Wagner & Robinson, 2001), with its single species, *T. laricifolium*, was shown from cpDNA restriction site data (Panero et al., 1999) to be sister to *Sphagneticola* from pantropical coastal areas of Mesoamerica and northern South America. It was earlier regarded as related to the Wedelioid generic complex (Eliasson, 1984).

Hawaiian Islands

This series of isolated islands has long attracted interest of island biologists, in part because of the extremely high level of endemism among angiosperms, ca. 90% at the specific level (Keeley & Funk, 2011). In addition to specific endemism, the archipelago harbors 34 endemic genera of angiosperms (Table 1), the most of any oceanic island system in the world. Furthermore, a comprehensive flora of the archipelago has recently been completed (Wagner et al., 1999), which for the first time has provided a consistent species concept for the many divergent plant lineages. This, in turn, has opened the way for modern investigations on patterns and processes within the endemic flora. A number of molecular phylogenetic studies, therefore, have already been completed on many of the endemic genera.

Despite strong molecular interest in general in the Hawaiian archipelago, four endemic genera have not yet been so investigated, and hence their status remains unchanged. *Dissochondrus* (Gramineae) with its single species, *D. biflorus*, is clearly related to the large genus *Setaria* and "perhaps congeneric" with it (O'Connor, 1999). Molecular studies have surveyed relationships among species of *Setaria* (Kellogg et al., 2009), as well as among genera of Paniceae (Giussani et al., 2001), but neither included samples of *D. biflorus*. It remains, therefore, a distinct endemic genus at this time. *Hibiscadelphus* (Malvaceae) contains seven species that are presumably holophyletic and closely related to *Hibiscus* (Lorence & Wagner, 1995). A morphological cladistic analysis has been completed on relationships among the endemic species but not including representatives of the diversity within *Hibiscus* (Funk & Wagner, 1995). Molecular studies have been completed on relationships among genera of Malvaceae (Pfeil et al., 2002, 2004), but no samples of *Hibiscadelphus* have been included. For the present, therefore, this Hawaiian genus remains endemic. *Neraudia* is a genus of Urticaceae with five closely related species (Wagner et al., 1999). The affinities of *Neraudia* are unclear at this time. Cowan (1949) suggested a tie to *Pouzolsia*. There have been two molecular phylogenetic studies in Urticaceae (Ueda et al., 1997; Monro, 2006), but no samples of *Neraudia* were included. *Touchardia*, also of Urticaceae, is a monospecific genus, suggested by Wagner et al. (1999: 1310) as possibly sharing "a common ancestor with *Urera glabra*." More studies obviously need to be done on these genera. *Urera laciniata* was sampled in a detailed molecular phylogenetic study of Urticaceae using ITS, *trnL-F*, plus morphology (Monro, 2006), but *Touchardia* was not included.

Another genus not investigated at the molecular level, but which has lost its endemic status as a result of morphological studies is *Pelea* (Rutaceae). The genus is nearly endemic to the Hawaiian Islands, with 48 species in the archipelago and only two in the Marquesas (Wagner et al., 1999). Morphological investigations have led to the submergence of *Pelea* into *Melicope* as a distinct section (Hartley & Stone, 1989). In the recent monograph on *Melicope*, Hartley (2001) lists section *Pelea* as consisting of 85 species distributed in Taiwan, Philippines, Borneo, Hawaii, Marquesas, and south to New Caledonia.

A number of other endemic genera in the Hawaiian archipelago have been investigated at the molecular level and shown to be holophyletic; hence, they have retained their endemic status. *Hesperomannia* (Compositae), with three species, used to be considered as being related to South American genera of Mutisieae (Bremer, 1994), but recent molecular phylogenetic studies with chloroplast *ndhF* sequences show affinities to Vernonieae (Kim et al., 1998). More specifically, based on *trnL-F*, *ndhF*, and ITS sequences, the genus remains holophyletic and ties to *Vernonia* from Madagascar and *Gymanthemum* from Africa (Keeley et al., 2007).

The monospecific *Hillebrandia* (Begoniaceae) is morphologically distinct within the family. The more recent and comprehensive molecular phylogenetic studies, using 5.8S, 18S, ITS, and *rbcL* (Clement et

al., 2004) show the genus to be sister to *Begonia* and cladistically basal within the entire family (these are the only two genera). Morphological characters (45) were also added to the ITS (including 26S and 5.8S) data (Forrest et al., 2005), and the branching pattern results for *Hillebrandia* were the same. It is estimated that this ancient lineage should be 51 to 65 million years old, predating the current islands by at least 45 million years, and posing an interesting biogeographic problem. Whether of South Pacific-Malesian or boreotropical origin, this relictual genus remains a distinct island endemic.

Labordia (Loganiaceae) is an endemic genus of 15 species closely related to *Geniostoma* (with 24 species) of the Pacific Basin (Wagner et al., 1999). Conn (1980) submerged *Labordia* into *Geniostoma* as a subgenus, but as Wagner et al. (1999) pointed out, Conn did not study the Hawaiian species. Hence, comprehensive morphological studies, therefore, still remain to be completed. At the molecular level, however, using chloroplast *ndhF* and *rbcL* sequences (Backlund et al., 2000), *Labordia* and *Geniostoma* are sister genera (only one species of each was analyzed, however, *L. tinifolia* and *G. rupestre*). Morphological cladistic analyses have been done among genera of Loganiaceae (Bremer & Struwe, 1992), but *Labordia* was not included. Perhaps most importantly, artificial crosses have been made among eight species of *Labordia* plus *G. rupestre*. All of the species of *Labordia* cross easily with each other (Motley & Carr, 1998), but none with *G. rupestre*. This provided good evidence that the genera should be maintained as distinct (for concurrence see Wood et al., 2007).

Lipochaeta (Compositae, Heliantheae) is traditionally regarded as an endemic genus of 20 species (Gardner, 1979; Wagner et al., 1999). The genus has been divided into two well-marked sections (Gardner, 1979), section *Aphanopappus* (14 species with $2n = 30$ and 5-merous disc florets), and section *Lipochaeta* (six species with $2n = 52$, and with 4-merous disc florets), with a close tie to *Wedelia* being mentioned (Gardner, 1979). Artificial hybridizations (Rabakonandrianina & Carr, 1981) between *Wedelia biflora* ($n = 15$), *W. trilobata* ($n = 28$), four species of section *Lipochaeta*, and six from section *Aphanopappus*, showed a high level of crossability between *W. biflora* and species of section *Aphanopappus* (both are at the same chromosome level), yielding 15II in meiosis, but also nearly sterile. The authors concluded that section *Aphanopappus* must have been derived from *Wedelia* from an ancestor similar to *W. biflora*. Section *Lipochaeta* probably also has come from crosses between two species of *Wedelia*, perhaps also *W. biflora* and an unknown $n = 22$ species (Rabakonandrianina, 1980). Subsequently, *Lipochaeta* sect. *Aphanopappus* has been transferred (Wagner & Robinson, 2001) completely into *Melanthera* (along with the genus *Wollastonia*). *Lipochaeta* as a genus, then, remains endemic to the Hawaiian Islands but with only six species. Molecular phylogenetic studies using cpDNA restriction site data (Panero et al., 1999) have examined intergeneric relationships among genera of subtribe Ecliptinae (Heliantheae), but with only few representatives of the genera here under discussion. *Lipochaeta* ties preferentially to *Wollastonia* (100% BS), but the two species involved, *L. integrifolia* and *Wollastonia biflora* were both later transferred into *Melanthera*, there forming a sister group to another clade that has *M. nivea* at its base. Samples of *Lipochaeta* in the restricted sense were not included. Chumley et al. (2000) published an abstract reporting more detailed molecular phylogenetic studies in these genera using ITS, but as species names for the genera are not given in this brief summary, it is difficult to evaluate the relationships reported.

Nothocestrum (Solanaceae) is an endemic genus of four species (Symon, 1999), earlier considered by Rock (1913) as related to *Athenaea* with seven species from eastern Brazil (Mabberley, 2008). In recent molecular phylogenetic studies of the entire family using *ndhF* and *trnL-F* sequences (Olmstead et al., 2008), *Nothocestrum* (*N. latifolium* and *N. longifolium*) joins in a sister group relationship to the monospecific *Tubocapsicum* (*T. anomalum*) from China. *Nothocestrum* remains an endemic island genus with affinities still uncertain.

Nototrichium (Amaranthaceae) is an endemic genus of three species (Lorence, 1996; Wagner et al., 1999). Hillebrand (1888) believed the generic affinities to lie with *Achyranthes*, and Lorence (1996) concurs. Molecular phylogenetic studies using *matK* (Ogundipe & Chase, 2009), *matK/trnK* (Müller & Borsch, 2005), and *rbcL* (Kadereit et al., 2003) all show a sister relationship with *Achyranthes*. Eliasson (2004) accepts the genus as endemic, and so it remains.

Pteralyxia (Apocynaceae) consists of two species, one restricted to Kauai (*P. kauaiensis*) and the other to Oahu (*P. macrocarpa*). Here again, the affinities of this genus are not terribly clear, perhaps with *Vallesia*, a tropical American genus of five species into which Hillebrand (1888) first described *P. macrocarpa* (as *V. macrocarpa*). Molecular phylogenetic analyses (Livshultz et al., 2007) using chloroplast regions (*trnL* intron, *trnL-F* spacer, *rpl16* intron, *rps16* intron, *matK*, and 3′ half of the *trnK* intron), plus 16 morphological characters, revealed *Pteralyx-*

ia (*P. kauaiensis*) to be sister to *Alyxia* (four species analyzed out of more than 100). These two genera were sister to *Plectaneia thouarsii*. Pollen studies by van der Ham et al. (2001) showed a close resemblance between *Pteralyxia* and *Alyxia*. *Pteralyxia* remains as an endemic genus at this time.

Schiedea (Caryophyllaceae) is a large endemic genus of 34 species (32 extant; Willyard et al., 2011), presumably having been derived (along with the related *Alsinodendron*) from an ancestor similar to *Arenaria*. A number of studies have been done on this large endemic complex of species (e.g., Wagner et al., 1995, 1999; Soltis et al., 1996; Weller et al., 2001; Kapralov & Filatov, 2006; Sakai et al., 2006). Much of the previous work is summarized in a recent monograph of the genus (Wagner et al., 2005). As shown in this treatment, with molecular phylogenetic analyses using nr ITS and ETS regions, plus morphology, *Schiedea* is holophyletic and highly supported (96% BS). The sister group consists of *Honckenya peploides* and *Wilhelmsia physodes*. Further molecular phylogenetic investigations by Willyard et al. (2011), using eight plastid and three low-copy nuclear markers, confirm these points as well as providing support for holophyly ("monophyly") of the many species. In context of the present study, there seems no doubt that *Schiedea* remains an endemic genus.

The two largest adaptively radiating generic complexes in Hawaii, the silverswords and lobeliads, have been investigated at the molecular level, and both are now known to contain different types of topological phylogenetic relationships. One dramatic example of evolution of endemic genera in oceanic islands is found in the silversword alliance of 28 species, consisting of *Argyroxiphium*, *Dubautia*, and *Wilkesia* (Compositae, Madieae). The three genera are nested as a holophyletic group within the other genera of Madiinae (Baldwin, 2003). A molecular analysis (ITS) among all species of the three genera reveals *Argyroxiphium* and *Dubautia* to be clearly holophyletic, but *Wilkesia* is derived from out of *Dubautia*, tying in a clade with *D. paleata*, *D. raillardioides*, and *D. latifolia*. Despite this topological position, so far no effort has been made to submerge *Wilkesia* formally into *Dubautia*, perhaps due to the extreme morphological divergence of the former (Carr, 1985) and its high conservation visibility.

The Hawaiian lobeliads are a huge holophyletic assemblage of ca. 126 species (Givnish et al., 2009), the largest adaptively radiated group on any oceanic island. Among the endemic genera are *Brighamia*, *Clermontia*, *Cyanea*, *Delissea*, *Rollandia*, and *Trematolobelia*. *Trematolobelia*, with four species, is shown from seven chloroplast regions to be sister to *Lobelia* sect. *Galeatella*, also nested among other endemic species of *Lobelia*. In a recent revision of *Trematolobelia*, Lammers (2009) points to this topological result and concludes that deciding on the proper status of this endemic genus must wait until the larger genus, *Lobelia*, can be carefully investigated and taxonomically evaluated. At present, therefore, *Trematolobelia* remains an endemic genus in the archipelago. Based on all molecular phylogenetic studies to date with chloroplast sequences (Givnish et al., 1995, 2009; Givnish, 1998; Antonelli, 2008), *Cyanea* and *Clermontia* are both holophyletic and sister to each other. Likewise, *Brighamia* and *Delissea* are sister taxa and both also holophyletic. These genera, therefore, retain their endemic island status. *Rollandia* has been recognized as a distinct endemic genus with eight species (Lammers, 1999; Wagner et al., 1999). Molecular phylogenetic studies, however, using chloroplast restriction site data (Givnish et al., 1995) reveal *Rollandia* to be a holophyletic unit but nested within *Cyanea*. As a result of the early availability of these data, Lammers et al. (1993) merged *Rollandia* into *Cyanea*, emphasizing that there is only one morphological feature that defines the former: the adnation of the staminal column to the corolla. In the face of molecular data, this was judged no longer sufficient to support generic recognition. *Rollandia*, therefore, disappeared as an endemic genus.

Munroidendron (Araliaceae) is a monospecific endemic genus closely related to, and possibly derived from, *Tetraplasandra* (also endemic with seven species; Lowry & Wood, 2000), *Reynoldsia* (six species, Samoa, Marquesas, Society Islands, one in Hawaii [*R. sandwicensis*]; Lowry, 1999), or *Gastonia* (11 species from eastern Africa, Malesia, and the Indian Ocean islands; Philipson, 1970). Relationships among these genera were studied at the molecular level by Costello and Motley (2001) using ITS1 and 2 and the non-transcribed spacer of nr 5S. *Tetraplasandra* is seen as holophyletic and substantiated as a good genus. The one species of *Gastonia* included, *G. rodriguesiana*, was designated as the outgroup and remains apart. *Munroidendron* (*M. racemosum*), however, ties as sister to *R. sandwicensis*, but only two of the six species were analyzed (the other being *R. verrucosa*). *Reynoldsia verrucosa* is the sister taxon to all other species of *Tetraplasandra* and *R. sandwicensis* and *M. racemosum*. The results suggested that most Hawaiian members of this complex are holophyletic, but with *Reynoldsia* showing "apparent paraphyly." This hesitation was

due to the limited sample of only two out of six species of the genus being analyzed plus some incongruence between trees for ITS and 5S-NTS. Costello and Motley (2001) suggested that all three genera could be combined into one genus, or alternatively, *R. sandwicensis* might be combined into *Munroidendron*. These same authors (Costello & Motley, 2007) again examined relationships among these genera using ITS, 5S-NTS, plus morphology (39 morphological and anatomical features), increasing the sample to include all species of *Tetraplasandra* (except for *T. lydgatei*), *Reynoldsia*, *Munroidendron*, and three species (four with morphological data) of *Gastonia*. With molecular data, *Munroidendron* ties again sister to *R. sandwicensis* with high (100% BS) support. With morphological data only, *Munroidendron* joins in a polytomy with all species of *Tetraplasandra*. The authors again sketch taxonomic alternatives to deal with these results, but the one they "prefer" is to treat as a separate genus the non-Hawaiian species of *Reynoldsia*, and *R. sandwicensis* and *Munroidendron* together as a single genus. This being the case, *Munroidendron* would become bispecific but still remain as an endemic genus. In parallel, however, Plunkett and Lowry (with associates) have examined broad generic relationships among genera of Araliaceae based on ITS (Plunkett et al., 2001), with *trnL-F* (Plunkett et al., 2004a, 2004b), and these also combined with ETS (Plunkett & Lowry, 2010). All results are summarized in Lowry and Plunkett (2010). The taxonomic approach they have taken has been to maximize holophyly and minimize nomenclatural change, which led to a very broad concept of the genus *Polyscias* to include *Munroidendron*, *Reynoldsia*, plus other taxa, in *Polyscias* subg. *Cuphocarpus*. *Munroidendron*, therefore, disappears from the endemic genera of the islands.

Some recent molecular phylogenetic investigations in the Hawaiian archipelago have provided inconclusive results regarding relationships, based mainly on limited sampling, and the genera, therefore, are still considered endemic. *Bobea* (Rubiaceae, Guettardeae), with four species, is shown with ITS sequences to be sister to *Guettarda* (Achille et al., 2006). Five chloroplast sequence regions (Bremer & Eriksson, 2009) were not particularly helpful for resolving intergeneric relationships among *Bobea* and 13 other genera. Darwin and Chaw (1999) suggested that on morphological grounds *Bobea* is "closely related to *Timonius* DC." *Kokia*, another endemic genus of Malvaceae, consists of four species of unclear generic affinities (Bates, 1999). Morphological relationships among the species have been analyzed by Funk and Wagner (1995). Generic affinities have been suggested to be with *Thespesia* and the segregate *Cephalohibiscus* (Fryxell, 1979; Funk & Wagner, 1995). Molecular studies using *K. drynarioides* only, with *ndhF* sequences (plus 11 morphological features; Pfeil et al., 2002) and in another study combined with ITS sequences (Seelanan et al., 1997), all reveal a strong tie as a sister group (99% BS) with the genus *Gossypioides* (*G. kirkii*), consisting of two species from tropical Africa and Madagascar (Mabberley, 2008). Species from these two genera were used as outgroups to root a low-copy number gene analysis of *Gossypium* (Álvarez et al., 2005), but this provided no additional insights on affinities of *Kokia*. *Remya* (Compositae, Astereae) is an endemic genus consisting of three species, presumably related to the large (ca. 100 species) Australasian *Olearia* (Wagner & Herbst, 1987). The only molecular phylogenetic study of *Remya* and relatives is by Karaman-Castro and Urbatsch (2009) using ITS sequences. Overall resolution, however, is poor, only showing *R. kauaiensis* and *R. mauiensis* to be holophyletic (100% BS) and placed in the "Austral Grade" with representatives of *Olearia* and 13 other genera.

In several other cases in the Hawaiian Islands, molecular investigations have shown endemic genera to be nested within continental progenitors, hence making the latter paraphyletic, but the authors in these cases have not yet taken formal taxonomic action. This same situation was described above in the endemic silversword genus *Wilkesia* (Compositae) that clearly nests within *Dubautia* (also endemic).

Broussaisia is the only species of Hydrangeaceae in the Hawaiian Islands, and it represents an endemic genus. It has an unusual berry-like fruit, an almost inferior ovary, styles that are connate (these features from Wagner, 1999), and is also polygamo-dioecious (Samain et al., 2010). It was suggested (Fosberg, 1939; Wagner et al., 1999) to be related to *Dichroa*, which has 12 species in Asia and Malesia (Mabberley, 2008). Molecular phylogenetic studies using *rbcL* (Soltis et al., 1995) and *rbcL* combined with *matK* and morphology (Hufford et al., 2001) show *Broussaisia* to nest with species of *Hydrangea* (and *Dichroa*). With five chloroplast regions (Samain et al., 2010), *B. arguta* is nested within *Hydrangea* and distinct from *Dichroa*, which also falls within *Hydrangea*. The authors conclude that "inclusion [in *Hydrangea*] of these genera [and six others] is suggested" (p. 598), but no formal action was taken. Even more recent investigations with six cpDNA regions (Xiang et al., 2011) show *Broussaisia* as sister

to a clade containing six other genera plus five other species of *Hydrangea*.

Isodendrion (Violaceae) is an endemic genus of four species, but one is already extinct and three are considered rare (Wagner et al., 1999). Morphological and cytological data give suggestions that the genus may be related to *Hymenanthera* and *Melicytus*. Molecular phylogenetic studies in Violaceae, using *rbcL*, *atpB*, *matK*, and 18S rDNA (Tokuoka, 2008), and with *I. laurifolium* and *I. longifolium*, show them nested within *Hybanthus*; however, no taxonomic changes were suggested by the author.

Platydesma (Rutaceae) is an endemic genus of four species with obscure generic affinities based on morphology (Stone et al., 1999). Molecular phylogenetic studies using ITS sequences (Harbaugh et al., 2009) show *P. rostrata* and *P. spathulata* to be nested in the center of *Melicope*, but sister to all the endemic Hawaiian species sampled, all formerly in *Pelea*. *Platydesma*, however, is hermaphroditic and morphologically divergent and has not been included in *Melicope* by Hartley (2001). It remains an endemic genus at this time.

Two endemic genera of Labiatae occur in Hawaii, *Haplostachys* with five species and *Stenogyne* with 20 species. A third genus, *Phyllostegia*, is nearly endemic with 32 species restricted to Hawaii (Wagner et al., 1999) and only one in Tahiti (*P. tahitensis*). All three genera belong to subfamily Stachydeae (Scheen et al., 2010). Molecular phylogenetic studies using *rbcL* and *trnL* intron, plus the nr 5S non-transcribed spacer region (Lindqvist & Albert, 2002; Lindqvist et al., 2003), have shown all three genera to be holophyletic, individually and as a complex, but all nested within *Stachys*, particularly close to relatives of the Pacific Coast of North America (Baldwin & Wagner, 2010). The Hawaiian complex appears highly derived, being in the uppermost clade of *Stachys*. Lindqvist and Albert (2002) suggest transfer of the three genera into *Stachys*, but they point out that specific epithets already exist in *Stachys* in the case of two species of *Haplostachys*, 16 of *Phyllostegia*, and eight of *Stenogyne*, which would require considerable nomenclatural changes. This is an excellent example of the basic problem that this present paper addresses. To eliminate paraphyly in *Stachys* requires that the authors submerge the island genera, which they would prefer to do, but the morphological distinctness of these genera, plus concern about changes of specific epithets, has caused them to hesitate. Further studies on relationships among these genera are obviously also needed.

Finally, a few molecular studies have shown progenitors to be paraphyletic, which has led to formal taxonomic action and loss of endemic status for the island genus. *Rollandia* (Campanulaceae) was one such case discussed above. Another case is *Alsinodendron* (Caryophyllaceae), recently treated as an endemic genus of four species (Wagner et al., 1999), but clearly related to *Schiedea*. Molecular phylogenetic studies (ITS and ETS, plus morphology), summarized in Wagner et al. (2005), show *Alsinodendron* to be nested within *Schiedea* as a holophyletic clade sister to *S. verticillata*. *Alsinodendron* is now viewed as making up two sections of *Schiedea*, *S. obovata* and *S. trinervis* forming section *Alsinodendron* and *S. viscosa* and *S. lychnoides* constituting section *Nothoschiedea*. It should be mentioned that artificial hybridizations have been successful between the two genera, but this is not necessarily unusual among genera of holophyletic alliances in oceanic islands (e.g., also among the silversword genera; Carr, 1995).

Madeiran Islands

The Madeiran archipelago consists of the largest island Madeira (725 km^2), plus Porto Santo and the Desertas, all more than 950 km west of Portugal in the eastern Atlantic Ocean. Madeira itself contains 1163 vascular plants, of which 113 (10%) are endemics (Press & Short, 1994), the same percentage also prevailing within the entire archipelago. Among the endemics in the archipelago are five endemic genera: *Chamaemeles* (Rosaceae, one species), *Monizia* (Umbelliferae, one species), *Musschia* (Campanulaceae, two species), *Parafestuca* (Gramineae, one species), and *Sinapidendron* (Cruciferae, four species).

Three endemic taxa continue to be regarded as good genera. *Chamaemeles*, represented by the sole species *C. coriacea*, is endemic to Madeira, Porto Santo, and Deserta Grande (plus on the small Doca Island nearby). Affinities of this genus are obscure (Press & Short, 1994), and no molecular investigations have to date been completed on the taxon. *Monizia* is also a monospecific genus endemic to Madeira and Deserta Grande (as well as on Selvagen Grande, of the Salvage Islands, located 300 km south of Madeira). Molecular phylogenetic studies (Downie et al., 2001), especially ITS sequences (Spalik & Downie, 2007), have continued to support its recognition as a distinct genus endemic to the archipelago. *Sinapidendron* is an endemic genus with three species confined to Madeira and one (*S. sempervivifolium*) to Deserta Grande in the Desertas Islands. *Sinapidendron angustifolium* and *S. frutes-*

cens have been included in a molecular phylogenetic study of tribe Brassiceae using ITS and *trnL* intron sequences (Warwick & Sauder, 2005), forming a highly supported (99% BS) trichotomy with *Erucastrum varium* in ITS and identity with *Diplotaxis assurgens* with *trnL*. With combined data, the two species of *Sinapidendron* form a holophyletic group, sister to *D. siifolia*. Earlier cpDNA restriction site data (Warwick & Black, 1993) for many of the same taxa also revealed *Sinapidendron* to be holophyletic.

Two endemic genera of the Madeiran archipelago, however, have been discovered to be nested within larger genera of continental regions. *Musschia* is an endemic genus confined to Madeira and Deserta Grande (plus Doca Islet). Both species are considered rare (Press & Short, 1994). Roquet et al. (2008) completed a broad survey of genera in Campanulaceae using ITS and *trnL-trnF*, and *M. aurea* was included in the analyses. Combined data gave good resolution in which *Musschia* ties with 100% BS support to *Campanula peregrina* and *C. primulifolia*. Called the "Musschia clade," the authors favored placing this and two other main clades within *Campanula*, but they emphasized the need for further investigations before such a decision might be taken (and combinations actually made). *Parafestuca* is a monospecific endemic genus confined to eastern and central Madeira. A morphological reexamination and new chromosome data suggested inclusion of this genus into *Koeleria*, and this has been further supported by nr TS and cp *trnT-F* sequences in this same investigation (Quintanar et al., 2006). Within tribes Aveneae and Poeae, *P. albida* ties best with species of *Koeleria* in the well-supported (99% BS) Koelerinae clade. A new combination has been formally made, but due to the specific epithet being occupied already in *Koeleria*, the new name *K. loweana* has been proposed. *Parafestuca* disappears, then, as an endemic genus in Madeira.

Robinson Crusoe Islands

Of 73 total angiosperm genera occurring in the Robinson Crusoe archipelago (Marticorena et al., 1998), 12 are regarded as endemic (Table 1), which yields a relatively high 16% level of generic endemism. Three of these genera, two of them monotypic, have not yet been examined with modern techniques and interpretations, and, therefore, remain accepted as good genera at the present time. *Juania* (Palmae) is scarce on Masatierra Island, being found scattered on the highest ridges (Stuessy et al., 1983). Several pre-molecular studies were done on aspects of morphology and anatomy of *J. australis* (Moore, 1969; Tomlinson, 1969; Uhl, 1969), but the generic affinities were not precisely determined. *Selkirkia* is a monospecific, endemic genus of Boraginaceae, and the family has recently had molecular attention (e.g., Långström & Chase, 2002). *Selkirkia*, however, was not included in the samples and analyses, perhaps due to its extreme rarity. *Megalachne* (Gramineae) consists of two endemic species, *M. berteroana* and *M. masafuerana*, both of which occur on both major islands of the archipelago (Baeza et al., 2002). The genus has at times been included within *Bromus* (Pilger, 1920; Skottsberg, 1921, 1953), but more recently it has been regarded as distinct but close to *Bromus* and *Festuca* (Matthei, 1986; Watson & Dallwitz, 1992) or *Metcalfia* and *Podophorus* (Soreng et al., 2001). Molecular studies have been done on *Bromus* (Saarela et al., 2007), but neither species of *Megalachne* was included.

The poorly known monospecific endemic genus *Yunquea* (Compositae, Cardueae) has recently been regarded as synonymous with *Centaurodendron* (Susanna & Garcia-Jacas, 2001) based on limited morphological data, but this species, known only from the virtually inaccessible peak El Yunque on Masatierra Island, has not been studied in any meaningful way, morphological or otherwise. Molecular surveys of subtribe Centaureinae have been accomplished (Garcia-Jacas et al., 2001; Susanna et al., 2011), but *Yunquea* has not been included.

Two genera have been investigated recently and submerged into other genera based primarily on other than molecular data. Based on morphological analyses, the monospecific genus *Rhetinodendron* (Compositae, Senecioneae; *R. berteroi*) was included into the larger endemic genus *Robinsonia* by Pacheco et al. (1985) but retained at the subgeneric level (Sanders et al., 1987). Subsequent ITS sequence studies (Sang et al., 1995) supported this taxonomic configuration. However, even more recent ITS studies, using a larger sample of species in the close continental relative *Senecio* (Pelser et al., 2007, 2010b), question this conclusion and suggest that *R. berteroi* might be more closely related to another group of species of *Senecio* in South America. Six combined chloroplast sequences (*trnL-trnL-F*, *psbA-trnH*, 3′*trnK*, 5′*trnK*, *ndhF*, and *psbJ-petA*) used by the same authors, however, show the genus to be holophyletic. Unfortunately, this all becomes sadly academic as introduced rats have destroyed the last known living specimen, and *R. berteroi* is now presumed extinct (Danton & Perrier, 2005).

Other genera have been investigated at the molecular level, and they remain acceptable in a cladistic context at this point. *Cuminia* (Labiatae)

with two species, based on *trnL/F* and ITS sequences, appears a good genus related to *Monardella* and *Minthostachys* (Trusty et al., 2004) or more broadly to the New World *Clinopodium* group (Bräuchler et al., 2010). The hybrid ×*Margyracaena* (Rosaceae) exists as a stabilized vegetatively reproducing intergeneric hybrid (as ×*M. skottsbergii*) between the native *M. digynus* and the introduced *Acaena argentea*, its origin documented by RAPD markers (Crawford et al., 1993). Its hybrid origin is likely to have occurred during historical times.

Two endemic genera, *Dendroseris* and *Robinsonia* (Compositae), have been shown to be nested within larger continental progenitors. *Dendroseris* is the largest endemic genus in the archipelago with 11 species. A member of the dandelion tribe, Lactuceae, it is a group of striking rosette trees 3–5 m tall, which many years ago were recognized as a distinct genus (Don, 1832). Divergence within the complex, however, is dramatic, such that Skottsberg at one point (1953) elected to recognize four genera within the complex. These species are now treated as belonging to three subgenera within one genus (Sanders et al., 1987), but there is no question that there is much morphological diversity. Molecular sequence studies using nrITS (Sang et al., 1994) have suggested *Dendroseris* to be holophyletic. This early result was confirmed by a much-expanded database and sampling within subtribe Sonchinae using ITS and chloroplast *matK* sequences (Kim et al., 2007). In this study *Dendroseris* was shown to be nested within the diverse and widespread genus *Sonchus*. Acting on these results, recently Mejías and Kim (2012) have made the formal combinations into *Sonchus*. Because many of the specific epithets in *Dendroseris* also exist within the large genus *Sonchus*, this required new names for five species. If this taxonomic perspective is adopted, then the largest endemic genus in the island disappears. The species, obviously, still remain endemic at the specific level.

The second largest genus of Compositae in the archipelago is *Robinsonia* (Senecioneae) with seven endemic species (including *R. berteroi*, originally in *Rhetinodendron* mentioned above). Molecular phylogenetic studies (Pelser et al., 2007, 2010a) have shown this genus to nest clearly within the very large *Senecio* that has more than 200 species in continental Chile alone (Cabrera, 1949) and perhaps 1200 throughout the world (Nordenstam, 2007). On the basis of these results, Pelser et al. (2010b) combined *Robinsonia* formally into *Senecio*. Due to so many epithets already pre-empted within the larger *Senecio*, four new names have been created. On the morphological level, *Robinsonia* is quite distinct in being a series of rosette dioecious woody trees 0.5–3 m tall.

Molecular investigations of *Centaurodendron*, another endemic genus of Compositae (Cardueae) with two species in the islands, using combined ITS, ETS, and plastid *trnL-trnL-F*, *rpl32-trnL*, and *ndhF* (Susanna et al., 2011), show it (*C. palmiforme* analyzed) to be nested within the broadly disjunct *Plectocephalus* (occurring in Ethiopia, North America, and South America) along with the South American *Centaurea cachinalensis* and *C. floccosa*. Despite this topological position, which renders *Plectocephalus* paraphyletic, Susanna et al. (2011) have retained *Centaurodendron* as an endemic genus with arguments for doing so based on morphological distinctness. This is the only example, of which we are aware, whereby the authors have taken such an explicit stand (cf. discussion on Susanna et al., 2011: 268–270).

Perhaps the most interesting genus in the archipelago is *Lactoris* (Lactoridaceae, consisting of *L. fernandeziana*), not only being an endemic genus, but also an endemic family, the only family of flowering plants restricted to a single oceanic island. Therefore, *Lactoris* has a double importance in conservation at both generic and familial levels, and it has been used as a rallying focus for plant conservation in the islands. There are perhaps 1000 plants existing on the higher difficult-of-access regions of Masatierra (Robinson Crusoe) Island (Crawford et al., 1994, 2001). Molecular sequence studies have shown *Lactoris* to nest within Aristolochiaceae near *Aristolochia* based on *rbcL* (Qiu et al., 1993), 18S rDNA (Soltis et al., 1997), and a 17-gene analysis (Soltis et al., 2011). If one were to accept these data and analyses and act on strict holophyly, the family Lactoridaceae would disappear from the island (and the world). The branch lengths involved with the phylogenetic reconstructions are very long, however, and this leaves some doubt as to the actual affinities of this genus (Stuessy et al., 1998). Additional molecular phylogenetic investigations (Wanke et al., 2006) using the chloroplastic *matK* and *trnK* show *Lactoris* to be the sister group to all other sampled Aristolochiaceae. Morphologically the family is quite distinct in its trimerous floral symmetry, zig-zag branching, and tetrad pollen (Zavada & Taylor, 1986; Sampson, 1995). A reasonable perspective at this time seems to be to retain *Lactoris* in a separate family but indicate a close relationship to Aristolochiaceae. It clearly belongs in Aristolochiales or Piperales, a position that is supported also by morphological and anatom-

ical data (Carlquist, 1990, 1992; Tobe et al., 1993; Tucker & Douglas, 1996; González & Rudall, 2001).

Somewhat surprisingly, a new endemic genus, *Nothomyrcia* (Myrtaceae), has just been added to the archipelago based on molecular phylogenetic studies. This genus, with its sole species, *N. fernandeziana*, is the dominant forest tree on Masatierra (Robinson Crusoe) Island and was previously included within *Myrceugenia*. Another endemic species of this genus, *M. schulzii*, also occurs in the archipelago but on the younger island of Masafuera. Recent molecular phylogenetic studies based on ITS, ETS, and four chloroplast markers (partial *trnK-matK*, *rpl32-trnL*, *trnQ-5′rps16*, and *rpl16*; Murillo-A. et al., 2012) have shown that *Myrceugenia fernandeziana* is very genetically divergent and falls outside all other known genera of Myrteae, being nearest to *Blepharocalyx*. If one acts taxonomically from these new data, a separate genus should be recognized under the existing name *Nothomyrcia* (Murillo-Aldana & Ruiz, 2011). This, then, represents a new endemic genus in the archipelago based on the new molecular data—a surprising reverse trend! It must be cautioned, however, that the most recent monographer of *Myrceugenia*, Landrum (1981a, 1981b), is not convinced that separate generic status for *M. fernandeziana* is warranted at this time based on morphological considerations (pers. comm.).

St. Helena

This single island of 121 km² sits in the South Atlantic Ocean some 1931 km west of the African continent. Due to this isolation, 49 endemic species of ferns and angiosperms have developed within a total flora of 320 species (Cronk, 2000). Among these occur 10 endemic flowering plant genera (all but two being monospecific): *Mellissia* (Solanaceae), *Nesiota* (Rhamnaceae), *Nesohedyotis* (Rubiaceae), *Trimeris* (Campanulaceae), and *Trochetiopsis* (Sterculiaceae, three species), plus five endemic genera of Compositae, *Lachanodes* and *Pladaroxylon* (Senecioneae), *Melanodendron* and *Commidendrum* (Astereae, the latter with four species), and *Petrobium* (Coreopsideae).

Several genera endemic to St. Helena have not yet been investigated molecularly, and for these, no change of status has been recommended. Lacking modern studies are *Nesiota* and *Trochetiopsis*, the latter with three species that sadly are now extinct in the wild (Cronk, 2000). Relationships among genera of Sterculiaceae have been examined with molecular data (Alverson et al., 1999; Wilkie et al., 2006), but the rare *Trochetiopsis* was not included.

Other genera have been investigated with new molecular markers, but no change in generic status has been suggested. *Mellissia* was submerged by Hunziker (1995, 2001) into *Withania* based on morphology. Molecular data with cp *ndhF* and *trnL-F* (Olmstead et al., 2008) suggest *M. begoniifolia* as sister to two species of *Withania*. *Mellissia*, therefore, is still regarded as a good endemic genus, and Fay et al. (2007) concur. The monospecific *Nesohedyotis* (*N. arborea*) was analyzed with up to five chloroplast regions (*rbcL*, *trnT-F*, *rps16*, *atpB-rbcL*, and *ndhF*) in a broad survey of subfamily Rubieae, tribe Spermacoceae (Bremer & Eriksson, 2009), and it falls in a large unresolved polytomy with representatives of 10 other genera of Rubioideae (such as *Mitracarpus*, *Psyllocarpus*, etc.). A survey using four chloroplast (*atpB-rbcL*, *petD*, *rps16*, and *trnL-F*) and two nuclear (ITS and 5S-NTS) DNA regions (Kårehed et al., 2008) within Spermacoceae revealed *Nesohedyotis* as sister (and basal) to *Oldenlandia tenuis* and *O. salzmannii* and the "Spermacoce clade," but this position received only weak support. In another analysis of three chloroplast sequences (*atpB-rbcL*, *rps16*, and *trnL-trnF*) in tribe Spermacoceae (Groeninckx et al., 2009), *Nesohedyotis* ties as sister to *Emmeorhiza*. With regard to *Petrobium*, Stuessy (1988) suggested placement into *Bidens*, but the molecular (ITS) data (Kimball & Crawford, 2004) do not support this. Ryding and Bremer (1992) were likewise against such a transfer on morphological and cladistic grounds. *Lachanodes* was submerged based on morphology into *Senecio* by Mabberley (1975), but molecular studies by Pelser et al. (2010a) using ITS/ETS and *ndhF*, *psbA-trnH*, 5′ and 3′*trnK*, and *trnL-F* intergenic spacers, regard it still as a good genus (as did Cronk, 2000). *Pladaroxylon* was very early placed as a section within *Lachanodes* (Endlicher, 1838), but Pelser et al. (2010a) treat the former as an independent genus. Mabberley (1975) also submerged *Pladaroxylon* into *Senecio*, but Jeffrey (1979) accepted the two genera.

New investigations in some cases have resulted in suggestions for change in generic status. *Melanodendron* and *Commidendrum* have been examined with molecular sequences (with ITS 1 and 2; Eastwood et al., 2004), and the phylogeny does "weakly suggest" that both "may be nested" (p. 81) within *Felicia*. The authors, however, believe that more sampling is required in *Felicia* and *Amellus* before relationships of the endemic island genera can be satisfactorily determined. The two genera are clearly related to each other in morphological and anatomical features (Cronk, 1987; Carlquist, 2001). *Trimeris* has been placed recently by Lammers (2011) into *Lobelia* (as

section *Trimeris*) based on morphological evidence, primarily due to his preference for keeping the latter genus large and not fragmented into many smaller, segregate genera. If recognized, these small genera would require many accompanying nomenclatural changes at the specific level. Molecular studies have been completed on Campanulaceae subfam. Lobelioideae (Antonelli, 2008) using *rbcL*, *ndhF*, and the *trnL-F* region, but *Trimeris* (i.e., as *L. suaevolifolia*) was not included.

Impact of New Data on Status of Genera Endemic to Oceanic Islands

The new analyses have led to several actions that impact taxonomic status of endemic genera in oceanic islands (Table 3). First, despite new investigations, the genera may still be recognized (i.e., a sister-group relationship is revealed causing no challenges to holophyly), in which case no taxonomic change has been instituted. Second, the genera may have been submerged already based on recent non-cladistic (or non-molecular) data and syntheses. Third, the genera have been revealed to be nested within a larger parental genus based on molecular phylogenetic studies. If this has happened, then the authors have acted on the information in one of three ways: (1) the genera are still recognized (i.e., no taxonomic modification); (2) the genera are submerged into the parental genera (or recommended that this be done) but without nomenclatural combinations being presented; or (3) the genera are submerged and combinations made formally. It is this last action, obviously, that truly eliminates the genera as endemic in the island or archipelago and creates an impact on conservation. These are taxonomic perspectives, of course, and no one is absolutely compelled to follow them. Furthermore, the taxa remain endemic species but not at the generic level.

Taking the detailed results from Table 3, we summarize in Table 4 the general impact of new data (especially molecular) and the cladistic interpretations on generic limits in oceanic islands. Sixty-four endemic genera continue to be accepted, mostly because they appear distinct (sister-group relationships) and not nested within any other genus. Seven genera have in recent years already been submerged based on non-molecular data and/or non-cladistic methods. A total of 29 genera have now been shown to nest within larger parental genera, the former of which would all disappear should concepts of absolute holophyly be applied. However, there is clearly some hesitation to eliminate some of these endemic genera as 15 are still recognized by the investigating authors. Six genera have been stated to belong more properly within the larger parents, but no formal transfers have yet been made. Finally, eight genera have disappeared from the ranks of island endemics by being combined into their parental stocks. It is important to remember, however, that there is no *absolute* requirement to submerge endemic island genera, no matter what the prevailing taxonomic or evolutionary context might be.

The overall impact of application of strict holophyly to avoid paraphyly for concepts of genera in oceanic islands is summarized in Table 5. The amount of change varies among island groups ranging from 29% to 43% (ignoring the Bonin Islands). The percent change in numbers of recognized endemic genera in the several oceanic islands/archipelagos examined would be 32% if all authors were to act taxonomically (and nomenclaturally) on the basis of classification using strict holophyly. That is, nearly one third of angiosperm genera endemic to these oceanic islands/archipelagos would disappear.

Perspectives on Holophyly and Paraphyly with Endemic Genera of Oceanic Islands

The problem of circumscription of genera in oceanic islands is simply one particular case of the challenge of recognizing genera worldwide (e.g., Barrett et al., 2005). There have been four basic approaches to delimiting genera during the past two or more centuries. First, a genus is recognized by divergence in characters, usually morphological, but supported and aided by other data from anatomy, embryology, palynology, cytology, DNA, and so on. Important in these comparisons is to seek gaps in character divergence such that species are grouped into genera based on characters they share with each other and that are not present in other species (or present to a different degree). Second, often ecological differences are used, based on geography, ecology, or both. The idea is that a genus may have entered a different adaptive environment or achieved a different adaptive norm from other groups of species. Widespread dispersal, obviously, can blur such distinctions. Third, genera usually do not cross easily with each other. This is a complex matter and usually not all species between two or more genera have been crossed either naturally or artificially, but in general, genera should cross with much more difficulty than should species. There is no cross known between families of angiosperms (Stuessy, 2009). Fourth, in a cladistic context, genera must be supported by synapomorphies (as with all taxa recognized in cladistic classification), and they must be holophyletic.

Table 3. Impact of molecular phylogenetic (or other evolutionary) studies on the taxonomic status of endemic genera of angiosperms in selected oceanic islands or archipelagos. Based on data in Ono and Kobayashi (1985), Wiggins and Porter (1971), Hansen and Sunding (1993), Press and Short (1994), Marticorena et al. (1998), Wagner et al. (1999), Cronk (2000), Danton et al. (2006), plus additional recent articles (cf. Table 2).

		Based on molecular phylogenetic studies, genus nested within a larger parental genus		
Genus still accepted	Genus submerged based on non-cladistic results	Genus still recognized	Genus submerged into parent (without combinations)	Genus submerged with combinations
Bonin Islands				
Dendrocacalia	*Boninia* (in *Melicope*)			
Canary Islands				
Aeonium *Aichryson* *Allagopappus* *Argyranthemum* *Bencomia* *Bethencourtia* *Bystropogon* *Dendriopoterium* *Dicheranthus* *Gesnouinia* *Gonospermum* *Ixanthus* *Kunkeliella* *Marcetella* *Monanthes* *Parolinia* *Schizogyne* *Spartocytisus* *Todaroa* *Vieraea*	*Heywoodiella* (in *Hypochaeris*) *Neochamaelea* (in *Cneorum*)		*Atalanthus* (as *Taeckholmia*) (in *Sonchus*) *Babcockia* (in *Sonchus*) *Greenovia* (in *Aeonium*) *Lactucosonchus* (in *Sonchus*) *Lugoa* (in *Gonospermum*) *Sventenia* (in *Sonchus*)	*Tinguarra* (in *Athamanta*) *Plocama**
Galápagos Islands				
Brachycereus *Jasminocereus* *Scalesia* *Trigonopterum*		*Darwiniothamnus* (in *Erigeron*) *Lecocarpus* (in *Melampodium*) *Sicyocaulis* (in *Sicyos*)		
Hawaiian Islands				
Argyroxiphium *Bobea* *Brighamia* *Clermontia* *Cyanea* *Delissea* *Dissochondrus* *Dubautia* *Hesperomannia* *Hibiscadelphus* *Hillebrandia* *Kokia* *Labordia* *Lipochaeta* *Neraudia* *Nothocestrum* *Nototrichium* *Pteralyxia* *Remya* *Schiedea* *Tetraplasandra* *Touchardia*	*Pelea* (in *Melicope*)	*Broussaisia* (in *Hydrangea*) *Haplostachys* (in *Stachys*) *Isodendrion* (in *Hybanthus*) *Phyllostegia* (in *Stachys*) *Platydesma* (in *Melicope*) *Stenogyne* (in *Stachys*) *Trematolobelia* (in *Lobelia*) *Wilkesia* (in *Dubautia*)		*Alsinodendron* (in *Schiedea*) *Munroidendron* (in *Polyscias*) *Rollandia* (in *Cyanea*)
Madeiran Islands				
Chamaemeles *Monizia* *Sinapidendron*		*Musschia* (in *Campanula*)		*Parafestuca* (in *Koeleria*)
Robinson Crusoe Islands				
Cuminia *Juania* *Lactoris* ×*Margyracaena* *Megalachne* *Nothomyrcia* *Selkirkia*	*Rhetinodendron* (in *Robinsonia*) *Yunquea* (in *Centaurodendron*)	*Centaurodendron* (in *Plectocephalus*)		*Dendroseris* (in *Sonchus*) *Robinsonia* (in *Senecio*)
St. Helena				
Lachanodes *Mellissia* *Nesiota* *Nesohedyotis* *Petrobium* *Pladaroxylon* *Trochetiopsis*	*Trimeris* (in *Lobelia*)	*Commidendrum* (in *Felicia*) *Melanodendron* (in *Felicia*)		

* *Plocama* has been increased in size to include *Aitchisonia*, *Choulettia*, *Jaubertia*, *Pterogaillonia*, and *Putoria* (Backlund et al., 2007).

Table 4. Summary of taxonomic impact of new molecular (or other evolutionary) studies on the status of endemic genera in oceanic islands or archipelagos.

			Based on molecular phylogenetic studies, genus nested within a larger parental genus		
Island or archipelago	Genera still accepted	Genera submerged based on non-cladistic results	Genera still recognized	Genera submerged into parent (without combinations)	Genera submerged with combinations
Hawaiian Islands	22	1	8		3
Canary Islands	20	2		6	2
Robinson Crusoe Islands	7	2	1		2
St. Helena	7	1	2		
Galápagos Islands	4		3		
Bonin Islands	1	1			
Madeiran Islands	3		1		1
Totals	64	7	15	6	8

The situation with regard to recognition of genera in oceanic islands, however, is somewhat different from other types of examples. Endemic genera in oceanic islands must evolve from immigrants that come from some source area, usually a continental region. Immigrants that are particularly successful in dispersing to islands often come from large and diverse continental genera, which have been evolutionarily successful due to a high ability for dispersal, adaptation, and character divergence. Once in the islands, the lineage may adapt and diversify further, either anagenetically if the islands offer limited ecological opportunities (Stuessy et al., 2006), in which case endemic genera do not develop, or cladogenetically resulting in adaptive complexes of related species. If divergence continues rapidly, these complexes may be so divergent that they are now regarded as genera distinct from their continental progenitors. Because this process is relatively rapid, the degree of molecular divergence may be less than morphological divergence. This may be because genes regulating adaptive features diverge more quickly than neutral or near-neutral markers, which are those that are often used to measure genetic divergence.

If cladistic analysis, using any type of comparative data, reveals that an island genus is nested within a larger paraphyletic progenitor, then there are three taxonomic options: (1) the genus can be submerged in the larger continental genus, hence removing the paraphyletic status of the latter; (2) one might elect to split the larger genus into as many smaller genera as needed to allow all lineages to become holophyletic; or, (3) one can simply recognize a large paraphyletic parental genus and a divergent holophyletic island genus.

The data reveal that those working in molecular phylogeny have so far not automatically acted to submerge island endemic genera into their continental progenitors. In fact, of the 29 cases listed in Table 4, only in eight have the actual transfers and combinations been made. This suggests a hesitation

Table 5. Numbers of endemic genera of angiosperms now accepted in seven oceanic islands/archipelagos in comparison with numbers of genera if strict cladistic holophyly (to avoid paraphyly) were applied taxonomically in all groups, showing percent change.

Island or archipelago	Number of endemic genera now accepted	Number of endemic genera resulting under strict holophyly	% change (loss) in number of endemic genera
Bonin Islands	1	1	0.0
Canary Islands	28	20	28.6
Galápagos Islands	7	4	42.9
Hawaiian Islands	33	22	33.3
Madeiran Islands	5	3	40.0
Robinson Crusoe Islands	10	7	30.0
St. Helena	10	7	30.0
Totals	94	64	31.9*

* Does not include Bonin Islands.

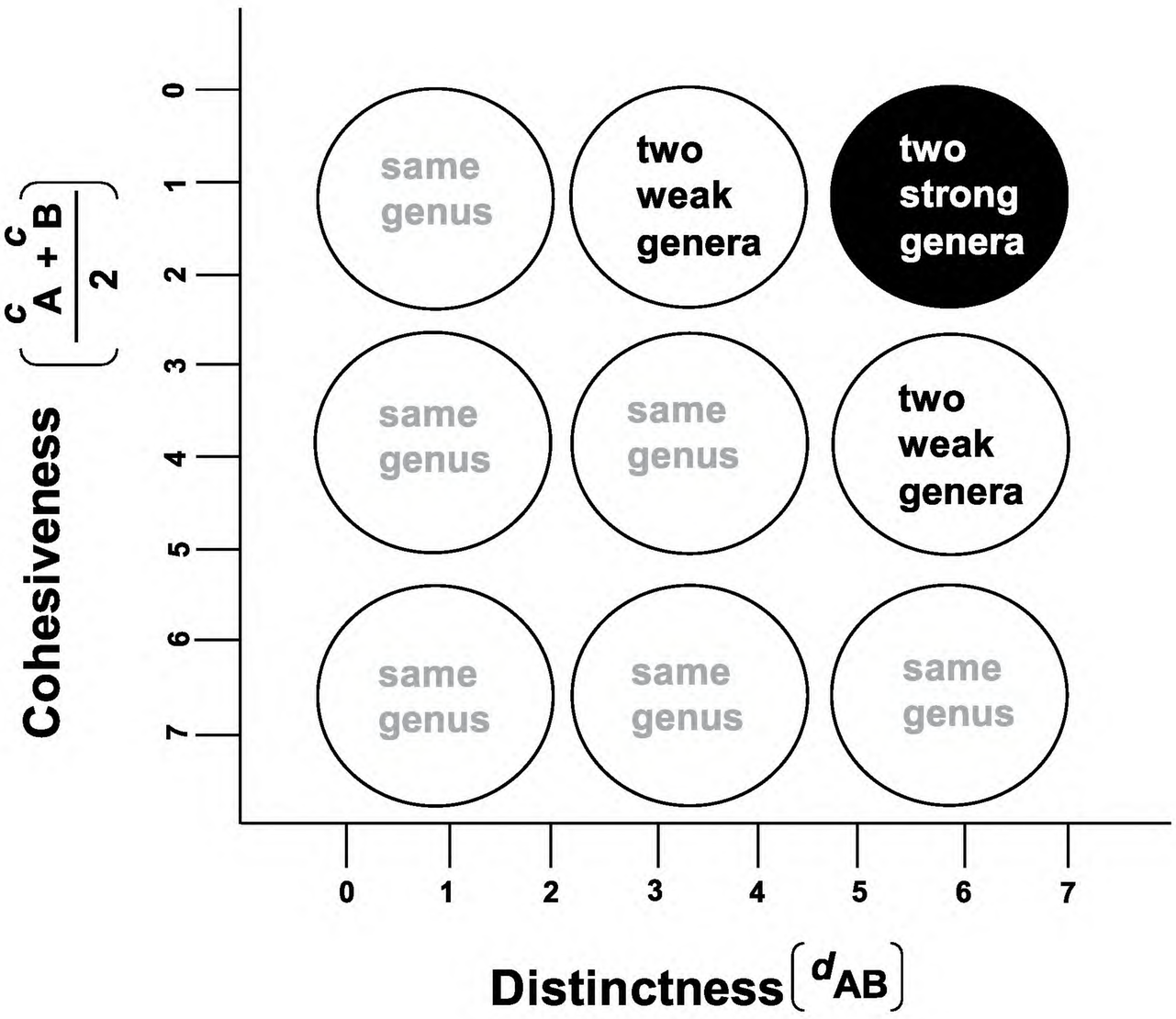


Figure 1. General relationship between cohesiveness and distinctness and the recognition of genera.

on the part of the participating investigators, perhaps for several reasons. First, it may be recognized that island taxa diverge rapidly due to strong directional selection and, using the generic level within the taxonomic hierarchy to emphasize this morphological divergence, may seem a logical alternative. Second, to some workers, simply because a group evolves from out of another group is no reason theoretically to have to submerge taxonomically the diverging unit back into the parental stock. Third, and perhaps most importantly, there may be hesitation to remove some of the endemic genera because in some cases they may be regarded as symbols of local (or national) pride or conservation importance in the island or archipelago. In the Robinson Crusoe Islands, for example, removal of the two largest endemic genera, *Dendroseris* (11 species) and *Robinsonia* (7 species), would reduce the conservation visibility to a considerable extent. As one specific example, the morphologically striking *D. litoralis*, nearly extinct in the wild, is grown in the village of San Juan Bautista as a source of island pride and identity. Conservation ethics do not (or should not) dictate scientific conclusions. But one has to wonder in this case if loss of endemic generic status of numerous morphologically divergent island taxa, simply to preserve the theoretical (cladistic) perspective of holophyly of continental progenitors, really makes any sense, scientific or otherwise.

These considerations bring us back to the question of how to recognize genera. A more sensible approach, in our view, is to evaluate generic status of a group not by simple reliance on holophyly, but by considering three dimensions taken together: (1) monophyly (in a broad sense), that is, not accepting polyphyly but admitting holophyly and paraphyly, (2) cohesiveness within the group, and (3) distinctness of the group from other known groups. Above all, the objective is maximum information content (high predictive quality) for our classifications, this being maximized by taking all evolutionary change into consideration, not which supports only the branching pattern.

Predictive quality (P) involving two genera, therefore, can be defined as:

$$P = ({}^{d}AB/{}^{c}A + {}^{c}B)/2$$

where d equals the distinctness between genera A and B, and c is the cohesiveness of genera A and B. Distinctness is measured by the character distance between the centers of the two groups (using a centroid method; Sneath & Sokal, 1973). Cohesiveness is the average character distance among group members (easily computed mathematically).

This statistic provides a yardstick for evaluation for generic recognition. Clear genera should give a predictive quality in the neighborhood of 5.0 or higher. Weak genera are at 1.0–2.0, and if P drops below 1.0, there is no argument for recognizing more than one genus. Figure 1 provides a visual scale that emphasizes that, as cohesiveness and distinctness increase, so does the argument for generic recognition.

Measuring predictive quality in this way is not phenetics. Phenetics is estimation of relationship by means of overall similarity, using as many characters as possible and without regard for evolutionary significance (Stuessy, 2009). In cladistics, one selects fewer characters of presumptive evolutionary import, uses them to assess relationships, and then uses only the synapomorphies to construct the branching diagram (cladogram). The main reason that cladistic classification is appealing is that it allows a classification to be converted conveniently (i.e., unambiguously) into a cladogram and the reverse. The cost of this, however, is to ignore part of the data (as uninformative) that we initially designated as evolutionarily significant. Phyletics (quantitative evolutionary classification) uses the same initial character information as cladistics, but all of it is assessed for purposes of developing predictive classifications because the autapomorphies that relate to divergence within lineages are also taken into account.

Quantitative morphological and/or genetic divergence is also not the entire picture for consideration with regard to generic recognition, especially that of endemic genera in islands. *Robinsonia*, for example, is nested within *Senecio* based on ITS sequence data (Pelser et al., 2007, 2010a), and new analyses by P. López (unpubl.) from data deposited in GenBank by Pelser et al., show a low *P* value of 1.43. This statistic of predictive quality shows that there is no greater molecular divergence of *Robinsonia* from species of *Senecio* than among other species within the genus. Based on these data and this statistic, there is no strong argument for keeping *Robinsonia* distinct from *Senecio*. Kim et al. (2007), in fact, made this same type of argument with respect to the large genus *Sonchus* and the incorporation of several generic segregates, including *Dendroseris* from the Robinson Crusoe Islands. These two endemic genera in the archipelago, however, are quite divergent morphologically despite low levels of measurable molecular divergence from progenitors using standard markers. *Robinsonia* contains species that are rosette trees and are dioecious, the morphological combination of which apparently does not occur within *Senecio* of continental South America. *Dendroseris* has species with different unusual morphologies, including being tall rosette trees, and one subgenus also being hummingbird pollinated, all very divergent morphologically from typical *Sonchus*. These morphological changes are, in fact, genetic and evolutionary responses of immigrant lineages into new oceanic island environments (Carlquist, 1974), and these can provide a basis for generic recognition.

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PARAPHYLY AND POLYPHYLY IN THE WORLDWIDE TRIBE RUBIEAE (RUBIACEAE): CHALLENGES FOR GENERIC DELIMITATION[1,2]

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ABSTRACT

On the basis of multidisciplinary studies on the tribe Rubieae, we contribute to the current discussion on paraphyly and supraspecific taxa that do not contain all descendant species of an ancestral clade. Rubieae belong to the large, predominantly tropical and woody family Rubiaceae and include possibly ≤ 1000 mostly temperate and herbaceous species with worldwide distribution. Our studies span distinctive groups throughout the tribe, consist of a maximum parsimony analysis of plastid *atpB-rbcL* and *rpL32-trnL* DNA sequences, and are summarized in a condensed strict consensus tree. A corresponding two-dimensional scheme illustrates alternative hypotheses for phylogenetic relationships among all major Rubieae clades identified. The small relictual genus *Kelloggia* Torr. in Benth. & Hook. f., formerly excluded from the Rubieae, is supported as a remnant of the ancestors of the tribe. *Didymaea* Hook. f. and *Rubia* L. represent early phylogenetic side lines. All other Rubieae form a large monophyletic crown group with the traditional genera *Asperula* L. being polyphyletic and *Galium* L. paraphyletic. Changes in the circumscription of these and other genera are thus inevitable. The necessity of accepting paraphyletic taxa as well as the positive and negative aspects of taxonomic splitting versus lumping within Rubieae are discussed. Additionally, lectotypes are designated for one section of *Asperula*—*Asperula* sect. *Dioicae* Airy Shaw & Turrill, typified by *A. conferta* Hook. f.—and for three sections of *Galium*—*Galium* sect. *Leiogalium* (DC.) Ledeb., typified by *G. sylvaticum* L.; *Galium* sect. *Lophogalium* K. Schum., typified by *G. multiflorum* Kellogg; and *Galium* sect. *Depauperata* Pobed., typified by *G. songaricum* Schrenk ex Fisch. & C. A. Mey.

Key words: *Asperula*, classification, evolution, *Galium*, *Kelloggia*, lumping, phylogeny, *Rubia*, Rubiaceae, Rubieae, splitting.

Important taxonomic questions that arise in the current field of biosystematics are (1) What principles should be used to build supraspecific taxa? (2) How should molecular and morphological evidence be balanced and incorporated? and (3) How should paraphyletic taxa that do not include all descendants from their common ancestral clade be dealt with? As a contribution to the current discussion of such key questions in biodiversity research, we present molecular and other evidence for relationships within the worldwide tribe Rubieae, subfamily Rubioideae of the giant family Rubiaceae. As a prerequisite for the correct circumscription, grouping, and naming of the clades recognized, we open with a brief survey of > 250 years of traditional taxonomy of the tribe and list all relevant genera and sections. Comments on key characters, ecogeographical differentiation, and internal structure of the accepted major clades follow. As general conclusions, we demonstrate positive aspects of careful splitting over excessive lumping within Rubieae and the paraphyletic nature of otherwise well-founded supraspecific taxa. In order to achieve such an improved classification, we prepare a subsequent, species-based, and fully documented phylogenetic study of the tribe.

TRADITIONAL CIRCUMSCRIPTION AND TAXONOMIC SUBDIVISION OF THE TRIBE RUBIEAE

The majority of what currently constitutes the tribe Rubieae was already assembled in the pre-Linnean literature as the Stellatae. This group was based mainly on its stipules transformed into leaflike elements arranged in whorls with the true, opposite leaves. Later, pluricolpate pollen grains were recog-

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nized as important for the circumscription of the tribe.

A brief historical survey of the internal taxonomic structure of Rubieae and an examination of Appendix 1 show that the genera *Asperula* L., *Crucianella* L., *Galium* L., *Rubia* L., *Sherardia* L., and *Valantia* L. were all recognized by Linnaeus in 1753, with *Cruciata* Mill. recognized by Miller in 1754. De Candolle (1830) formalized the Stellatae as a tribe of Rubiaceae, accepted most of the older genera, and partly subdivided them into infrageneric but unranked groups. Subsequently, these groups were formalized as sections within *Asperula* (*Asperula* sect. *Cynanchicae* (DC.) Boiss.) and within *Galium* (*Galium* sect. *Aparine* W. D. J. Koch, *Galium* sect. *Cruciata* W. D. J. Koch, *Galium* sect. *Eugalium* (DC.) W. D. J. Koch (= *Galium* sect. *Galium*), *Galium* sect. *Leiogalium* (DC.) Ledeb., *Galium* sect. *Platygalium* (DC.) W. D. J. Koch, and *Galium* sect. *Vaillantioides* W. D. J. Koch).

Intensified studies on the floras of southeastern Europe and southwestern to central Asia in the first half of the 19th century led to the description of the additional genera *Callipeltis* Steven, *Mericarpaea* Boiss., and *Microphysa* Schrenk, plus new sections of *Asperula*: *Asperula* sect. *Cruciana* Griseb., *Asperula* sect. *Glabella* Griseb., *Asperula* sect. *Hylaea* Griseb. (later transferred to *Galium* as *Galium* sect. *Hylaea* (Griseb.) Ehrend.), and *Asperula* sect. *Phuopsis* Griseb. (later elevated to genus as *Phuopsis* (Griseb.) Benth. & Hook. f.); and of *Galium*: *Galium* sect. *Aparine*, *Galium* sect. *Kolgyda* Dumort., *Galium* sect. *Leiogalium*, *Galium* sect. *Microgalium* Griseb., and *Galium* sect. *Thliphthisa* Griseb. (later transferred to *Asperula* as *Asperula* sect. *Thliphthisa* (Griseb.) Ehrend.). In the second half of the 19th century the newly described genus *Didymaea* Hook. f. (Central America) was included within the Rubieae in spite of its typical Rubiaceae stipules, but with pluricolpate pollen grains. During the same period we register the publication of the genera *Relbunium* (Endl.) Benth. & Hook. f. (mostly in South America, now part of *Galium* sect. *Platygalium* s.l.) and *Leptunis* Stev. (Southwest to Central Asia, now an annual member of *Asperula*), as well as *Galium* sect. *Aparinoides* (Jord.) Gren., *Galium* sect. *Leptogalium* Lange in Willk., and *Galium* sect. *Trachygalium* K. Schum. Major additions of supraspecific Rubieae taxa in the 20th century were *Asperula* sect. *Dioicae* Airy Shaw & Turrill and *Asperula* sect. *Hexaphylla* Ehrend., as well as *Galium* sect. *Cymogalia* Pobed., *Galium* sect. *Depauperata* Pobed., *Galium* sect. *Jubogalium* Ehrend., and *Galium* sect. *Orientigalium* Ehrend., and the generally neglected North American genus *Bataprine* Nieuwl. (now part of *Galium* sect. *Platygalium* s.l.). *Kelloggia* Torr. in Benth. & Hook. f., a genus with two species (in East Asia and North America), has been considered a member of the tribe Paederieae (Robbrecht, 1988; Nie et al., 2005) because it has typical Rubiaceae stipules and tricolpate pollen grains; only Robbrecht and Manen (2006) have recently included it into the Rubieae (see below).

In retrospect and in addition to the references cited in Appendix 1, traditional contributions toward supraspecific taxa within Rubieae were made available on a worldwide basis by de Candolle (1830) and Schumann (1897); for Europe, the Mediterranean, and southwestern Asia by Koch (1843), Grisebach (1844), Boissier (1875), Ehrendorfer (1975), and Ehrendorfer et al. (1976, 1982, 2005); for Russia and Central to East Asia by Ledebour (1844), Shishkin et al. (1958), Jelenevsky et al. (2003), and Chen and Ehrendorfer (2011); and for the Americas by Dempster and Stebbins (1968) and Dempster (1980, 1982, 1990).

DNA Sequences and Phylogenetic Relationships among Taxa of Rubieae

The first analytical DNA studies on members of the tribe Rubieae date back to the 1990s: Ehrendorfer et al. (1994), Manen et al. (1994), Natali and Manen (1995), and Natali et al. (1995, 1996). Particular attention has been given to the *atpB* promoter region by Manen and Natali (1995, 1996) and Manen (2000). Further studies have concentrated on the genus *Kelloggia* (Nie et al., 2005) or have gone beyond the tribe (Bremer & Manen, 2000; Robbrecht & Manen, 2006). Recently, more extended and detailed analyses within the tribe have been conducted by Soza and Olmstead (2010a, 2010b) and Ehrendorfer et al. (2011, and in prep.). Using different plastid markers, these studies support each other and allow for an increasingly improved recognition of all major Rubieae clades. Their circumscription and relationships can be much more clearly demonstrated now (Figs. 1, 2) than was possible before, on traditional morphological, karyological, and ecogeographical evidence alone.

All available DNA-supported phylogenies place the disjunct genus *Kelloggia* (clade I, Figs. 1, 2) with two species (*K. chinensis* Franch., eastern Asia; *K. galioides* Torr., western North America) at the base of the traditional Rubieae, in spite of the presence of a plesiomorphic 50-bp sequence in the *atpB* promotor region, characteristic for subfamily Rubioideae, but deleted in all other Rubieae (Nie et al., 2005). Outgroups of the tribe from all remaining Rubiaceae–

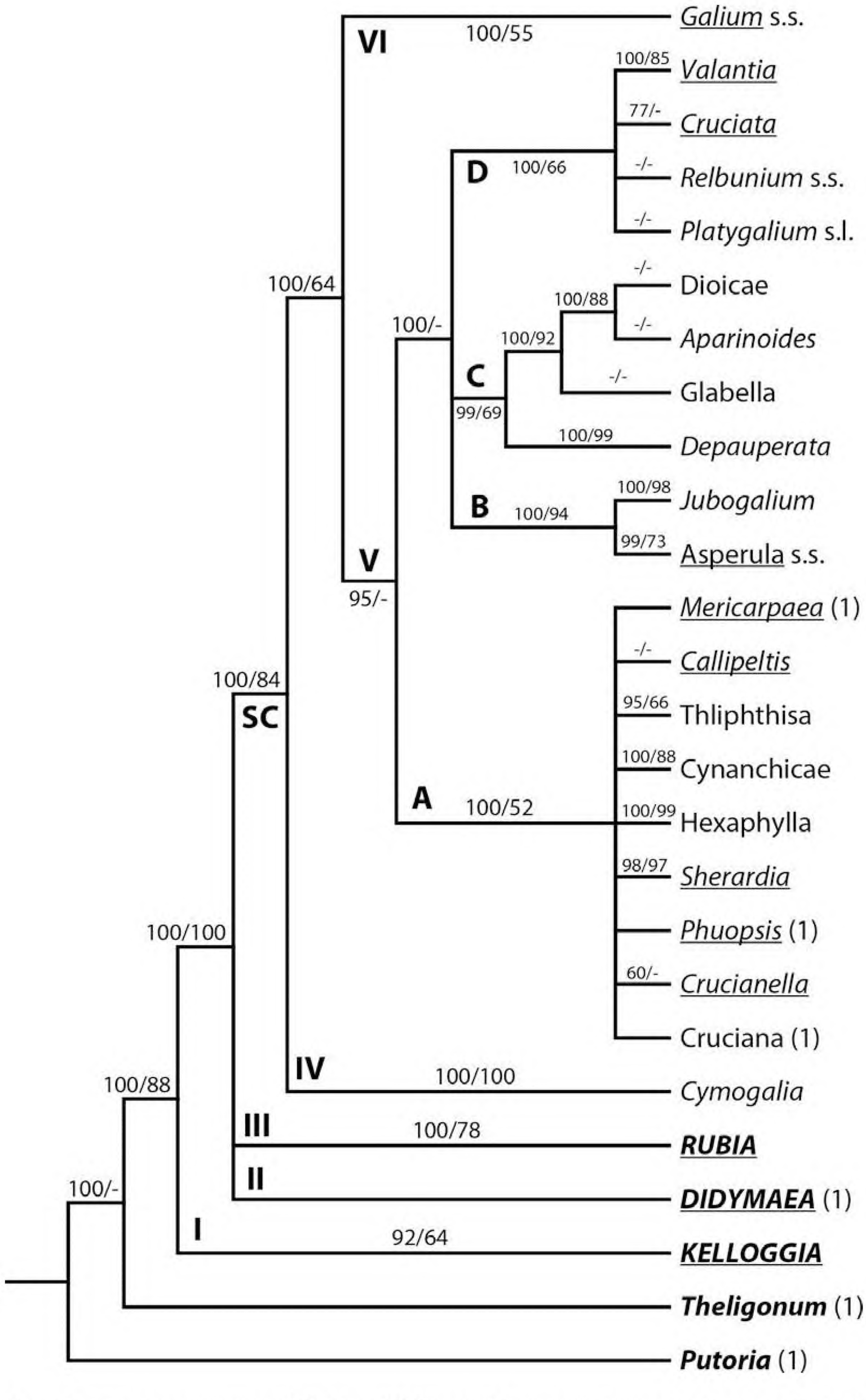


Figure 1. Condensed strict consensus tree of Rubieae (Rubiaceae) from a maximum parsimony analysis of the plastid *atpB-rbcL* and *rpL32-trnL* intergenic sequences of 254 worldwide accessions and 167 species, representing currently accepted 12 genera in Rubieae (underlined) and major sections within *Asperula* L. and *Galium* L., together with two outgroup genera (*Putoria* Pers. and *Theligonum* L.). Numbers on branches refer to posterior probabilities followed by bootstrap values. The main clades of Rubieae are marked with numerals: I (*Kelloggia* Torr. in Benth. & Hook. f. = Kelloggiinae), II + III (*Didymaea* Hook. f. + *Rubia* L. = Rubiinae) and SC (superclade *Galium* s.l.) with IV, VA–D, and VI. Sections published under *Asperula* are in normal script without italics; sections under *Galium* are in italics; accepted genera of Rubieae are in boldface capitals and underlined, outgroup in boldface, lowercase, and not underlined. Clades represented by only one accession are marked "(1)." Publication of the underlying detailed and fully documented plastid DNA phylogeny of all accessions studied is in preparation.

Rubioideae and in increasing phylogenetic distance are the morphologically strongly deviating genera *Theligonum* L. (Theligoneae) and *Putoria* Pers. (= *Plocama* Aiton s.l., Putorieae). Bremer and Eriksson (2009) estimate the divergence of the Rubieae (including *Kelloggia*) from *Theligonum* at ca. 28.6 Ma (million years ago; Late Oligocene), and Nie et al. (2005) at ca. 22.8 Ma (Early Miocene). Within the

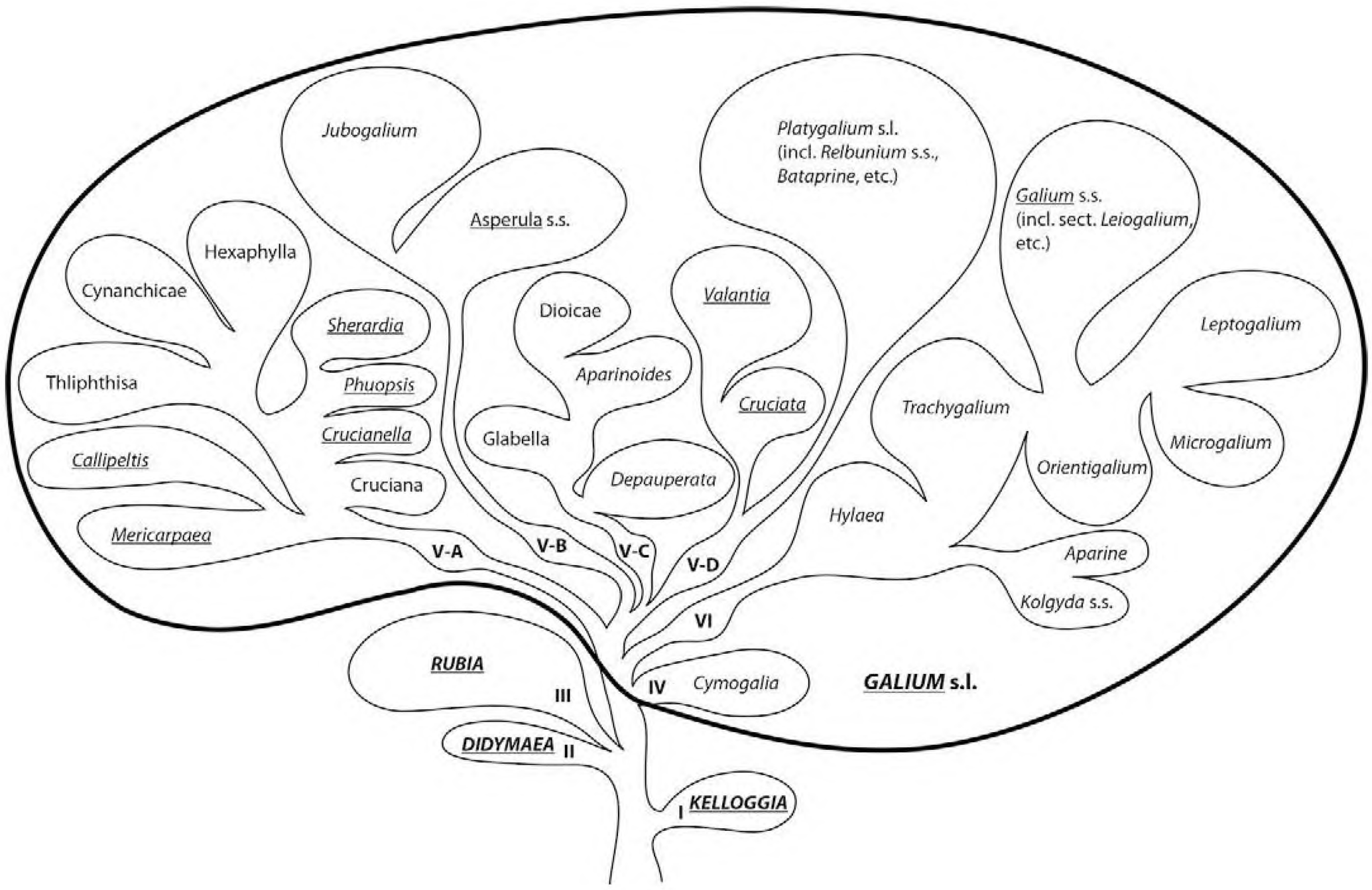


Figure 2. Proposed phylogenetic classification of Rubiaceae–Rubieae with the alternative hypotheses of lumping versus splitting. Lumping: all clades within the thicker encircling line become a single large genus *Galium* L. s.l.; only *Rubia* L., *Didymaea* Hook. f., and *Kelloggia* Torr. in Benth. & Hook. f. remain as segregate genera. Splitting: all separately named clades within IV and VA–D are recognized at a generic level, whereas the single clade VI corresponds to *Galium* s. str. Names currently recognized as genera are underlined. Sections traditionally recognized within *Asperula* L. are in normal script; sections within *Galium* are in italics. The numerals I–VI correspond to clades I–VI in Figure 1, with V including the species groups A–D. The thick, encircling line demarcates the superclade SC (including clades IV–VI) seen in Figure 1.

entire family, *Kelloggia* is the first clade to exhibit the apomorphy of hooked bristles on its dry mericarps, a most efficient character for fruit dispersal. This character becomes widespread within the rest of the tribe. Except for its true, inconspicuous stipules and its tricolpate pollen grains (plesiomorphic characters in the family), *Kelloggia* corresponds perfectly with other Rubieae. It qualifies as close to hypothetical ancestors of the tribe and definitely should be included, possibly as a subtribe Kelloggiinae (as already proposed by Robbrecht & Manen, 2006). A problem emerges if *Kelloggia* is not regarded as sister but rather as a surviving relic of Rubieae ancestors. This would make Keloggiinae paraphyletic from the standpoint of cladistics because the subtribe then should include all of its increasingly apomorphic extant descendants (cf. Schmidt-Lebuhn, 2011: fig. 2, X+C vs. A, B).

Didymaea (clade II in Figs. 1, 2), a small Central American genus of ca. 14 species, and *Rubia* (clade III in Figs. 1, 2), a Eurasian to African genus of ca. 80 species, partly retain typical plesiomorphic stipules. However, most taxa of *Rubia* already exhibit the innovation of conspicuous apomorphic, leaflike stipular elements in whorls with true leaves. Instead of tricolpate pollen, both genera develop pluricolpate pollen grains and share fleshy mericarps, both further apomorphic characters in the tribe. These two disjunct genera form a strongly supported superclade in most phylogenies (e.g., in Soza & Olmstead, 2010a) and apparently have diverged (or budded) early from the main line of the tribe. They can be regarded as subtribe Rubiinae s. str. of Rubieae.

The remaining ca. 900 species of Rubieae form a worldwide superclade (labeled SC in Fig. 1) and should be designated as a third subtribe based on *Galium* s.l. This superclade includes six highly supported clades identified as IV, VA–D, and VI (Figs. 1, 2). With the exception of the newly resolved clade IV, Soza and Olmstead (2010a) recently have also found all these clades independently and to be well supported by other DNA markers. Figures 1 and 2 illustrate this main result of our plastid DNA analyses: there is clear evidence that *Asperula* (appearing independently in clades VA–C) and *Galium* (rooted in clade IV and reappearing in VB–D and VI) are polyphyletic or paraphyletic in their present, traditional, and artificial circumscription.

The most basal branch of the superclade (SC) is clade IV (Figs. 1, 2) with the monotypic *Galium* sect. *Cymogalia*. It consists only of *G. paradoxum* Maxim., whose DNA has not been studied before. This is a sylvicolous perennial with a disjunct occurrence in the Urals (western Russia and northwestern Kazakhstan), whereas its main distribution is in eastern Asia. The inflorescence and hooked mericarp bristles of *Galium* sect. *Cymogalia* are remarkably similar to *Kelloggia*, but it deviates by having whorls of two larger leaves and two smaller leaflike stipules, both with a plesiomorphic camptodromous venation and by the apomorphic rotate (not funnel-shaped) flowers. One could interpret *Galium* sect. *Cymogalia* as a relic of an ancestral paraphyletic group that has given rise to the remaining, more apomorphic Rubieae clades VA–D and VI (see Fig. 2).

Clade VA (Figs. 1, 2), centered in southwestern Asia and the Mediterranean, has been recognized since the report published by Natali et al. (1995) as the *Sherardia* clade and was confirmed as clade IV by Soza and Olmstead (2010a). This group is mainly characterized by an elongate corolla tube with five to four lobes and usually glabrous, dry mericarps. Traditionally, these taxa have been relegated to separate genera, to *Phuopsis*, *Sherardia* (both with one species), and *Crucianella* (ca. 35 species), and to several sections in *Asperula*. This variability ranges from the rather plesiomorphic *Asperula* sect. *Cruciana* (ca. 10 species) with funnel-shaped and 5-merous flowers to the increasingly apomorphic *Asperula* sect. *Cynanchicae* ($\leq$ 80 species), *Asperula* sect. *Hexaphylla* (ca. 12 species), and *Asperula* sect. *Thliphthisa* (ca. 24 species) with 4-merous flowers, the latter even with partly rotate corollas. It is surprising that, according to their plastid DNA data, the extremely aberrant and apomorphic, annual, and autogamous genera *Callipeltis* (three species) and *Mericarpaea* (one species) are only loosely related to each other but clearly belong to clade VA (Figs. 1, 2). Both have inconspicuous rotate flowers, with *Callipeltis* characterized by enlarging inflorescence bracts that favor anemochorous dispersal and *Mericarpaea* distinguished by the hooked bristles on the fruits. Both genera are centered in southwestern Asia and suggest an unexpected, morphologically dramatic phylogenetic divergence within clade VA. Soza and Olmstead (2010a) came to similar results with *Callipeltis* but had no material of *Mericarpaea*. No DNA analyses are available yet from some southwestern to central Asiatic taxa of the traditional genus *Asperula*, that is, the perennials of *Asperula* sect. *Oppositifolia* Shishk ex Schönb.-Tem. (ca. 20 species) and four annual species that include the former genus *Leptunis* (Ehrendorfer et al., 2005). It is likely that they, too, group with clade VA.

Another unexpected relationship of clades, well supported by our DNA data, is encountered in group VB (Figs. 1, 2), where the traditional *Asperula* s. str. and *Galium* sect. *Jubogalium* (the latter not studied before) form highly supported sisters (100% bootstrap). *Asperula* s. str., a small group with about four species, is characterized by involucrate capitula of funnel-shaped flowers and includes perennial (*A. taurina* L.) and annual (*A. arvensis* L., etc.) taxa centered in the southeastern Mediterranean and southwestern Asiatic region. The more speciose *Galium* sect. *Jubogalium* (ca. 13 species) with rotate flowers and a particular truncate ovary structure and indumentum includes more xerophytic perennial and annual taxa, which extend from southeastern Arabia and Southwest Asia throughout the entire Mediterranean region (Ehrendorfer, 1958).

Even more heterogeneous and mostly perennial is group VC (Figs. 1, 2), partly recognized already by Manen et al. (1994) as their *Asperula* sect. *Glabella* clade and verified by Soza and Olmstead (2010a) as clade V. At its base we find *Galium* sect. *Depauperata* s. str., an aberrant group of three annual species with whorls of two leaves and two smaller leaflike stipules and fruits with uncinate bristles, ranging from central Asia to western North America. The core of clade VC consists of the mesophilous and strongly disjunct *Asperula* sect. *Glabella*, traditionally composed of ca. 13 species and extending from the Mediterranean (e.g., *A. laevigata* L.) to eastern Asia (i.e., *A. maximoviczii* Kom. $\equiv$ *G. maximoviczii* (Kom.) Pobed. on the Asiatic mainland and *A. trifida* Makino in Japan). *Asperula* sect. *Glabella* is closely linked to the also perennial but hygrophilous *Galium* sect. *Aparinoides*, quite speciose with ca. 20 worldwide distributed species (Puff, 1974). This group occurs throughout the Northern Hemisphere from North America (e.g., *G. obtusum* Bigelow) to Eurasia (e.g., *G. palustre* L.) and reaches the Arctic (*G. trifidum* L.), Africa, and southern South America (e.g., *G. antarcticum* Hook. f.). It is remarkable that the analyzed plastid DNA of *Galium* sect. *Aparinoides* has been found to be nearly identical with that of the morphologically quite distinct dioecious *Asperula* sect. *Dioicae* in Australia and New Zealand (22 species). Recent and still unpublished studies (2014) have supported these close affinities.

Clade VD (Figs. 1, 2) has been recognized already by Manen et al. (1994) and was designated herein as the *Cruciata* clade. This species group includes a large portion of the traditional genus *Galium*, in particular *Galium* sect. *Platygalium* s.l. and its

numerous relatives (as verified and greatly extended by Soza & Olmstead, 2010a, 2010b), possibly with > 250 species. Generally, members of clade VD are characterized by whorls of two leaves and only two similar leaflike stipular derivatives, all not rarely with three (or even more) parallel main veins. Its more plesiomorphic core (*Galium* sect. *Platygalium* s. str.) consists of perennial taxa with closed thyrsoid inflorescences (terminating in a flower), rotate 4-lobed hermaphroditic flowers without an involucrum of bracteoles, and dry mericarps often with hooked bristles or various hairs. Corresponding taxa have a worldwide distribution, from Eurasia to Africa, Australia, and New Zealand (Thompson, 2009) and the Americas. No DNA sequences are yet available for the monotypic genus *Microphysa* with *M. elongata* (Schrenk) Pobed. (≡ *Asperula elongata* Schrenk) endemic to central Asia, but from its morphology and similarity to *G. rubioides* L. it is very likely that it represents only an aberrant taxon within *Galium* sect. *Platygalium* (Chen & Ehrendorfer, 2011). The data presented by Soza and Olmstead (2010b) demonstrate that nearly all New World clades of group VD have originated by evolutionary budding (Hörandl & Stuessy, 2010) and divergent evolutionary radiation from a little-changed ancestor similar to *G. oreganum* Britton. This extant species from northwestern California to western Oregon and Washington is a plesiomorphic member of *Galium* sect. *Platygalium*. In contrast, the derived and mostly more apomorphic clades 1 to 9, as proposed by Soza and Olmstead (2010b: fig. 2), vary from perennial to annual, from hermaphroditic to polygamous and dioecious, from pedicillate and bare to sessile flowers with bracteolate involucra (*Relbunium*, sensu Ehrendorfer, 1955; Dempster 1982, 1990), and from dry mericarps with hooked to straight hairs (*Galium* sect. *Lophogalium*) to glabrous and repeatedly even to fleshy fruits (*Bataprine*, Californian taxa: Dempster & Stebbins, 1968; *Relbunium*, Central to South American taxa: Ehrendorfer, 1955). In many cases morphologically circumscribed New World groups are difficult to reconcile with the clades suggested by the DNA data (Soza & Olmstead, 2010a, 2010b). Creating supraspecific taxa without the near-ancestral *G. oreganum* would again result in problems with paraphyly.

In contrast to *Galium* sect. *Platygalium* s.l. with closed inflorescences and terminal flowers, the clade VD (Figs. 1, 2) also includes the early recognized and clearly related smaller genera *Cruciata* (ca. 12 species, western Eurasia) and *Valantia* (ca. seven species across the Mediterranean region). They are characterized by apomorphic, open inflorescences that have only lateral floriferous cymes and vegetative apical growth. Both genera include perennials and annuals, and both exhibit increasing specializations with respect to fruit dispersal (Ehrendorfer, 1965).

The last and also quite speciose element of the superclade SC is clade VI (Figs. 1, 2). It corresponds to the *Galium* sect. *Galium* clade of Manen et al. (1994), has been fully supported most recently by Soza and Olmstead (2010a, their clade III), and corresponds to our *Galium* s. str. (Figs. 1, 2). It includes the type species of the genus (*G. verum* L.) in *Galium* sect. *Galium* and species from about eight other sections, encompassing > 200 species. Morphologically, *Galium* s. str. is characterized by whorls of leaves and leaflike stipular derivatives with more than four (≤ 12) elements on well-developed stems, by the single main vein to the leaf, the terminal and closed thyrsoid inflorescences, rotate (only rarely funnel-shaped), 4-lobed hermaphrodite flowers, and dry mericarps, sometimes with hooked bristles, various hairs or often glabrous.

The natural distribution of *Galium* s. str. is concentrated in the Old World, with centers of diversity in eastern and southwestern Asia but also in Africa. Apart from introduced taxa, only a few species have reached North America (e.g., *G. asprellum* Michx., *G. concinnum* Torr. & A. Gray, *G. mexicanum* Kunth, and *G. triflorum* Michx. from *Galium* sect. *Trachygalium*, and possibly *G. aparine* L. s.l. from *Galium* sect. *Aparine*), but none in South America (*G. surinamense* Dempster is highly doubtful).

The internal relationships of *Galium* s. str. (clade VI, see Fig. 2) are complex. With eastern Asian members of *Galium* sect. *Hylaea*, particularly *G. hoffmeisteri* (Klotzsch) Ehrend. & Schönb.-Tem. ex R. R. Mill, clade VI is morphologically clearly linked to *G. paradoxum* (only species of clade IV, *Galium* sect. *Cymogalia*) by remarkable morphological similarities in life form, inflorescence, and hooked bristles on its dry mericarps. This linkage at the very base of the SC superclade and *Galium* s.l. (Figs. 1, 2), together with the available DNA data, suggest that *Galium* sect. *Hylaea*, limited with about eight species to Eurasia, is relatively plesiomorphic and occupies a basal and paraphyletic position within clade VI (see Fig. 2) because all other sections of this clade presumably have originated from similar ancestors. *Galium* sect. *Trachygalium* is closely linked to *Galium* sect. *Hylaea* by *G. triflorum*, which combines its creeping rhizomes, leaf morphology, and hooked fruit bristles with partly retrorsely aculeolate stems. The latter characterizes *Galium* sect. *Trachygalium*, a diverse and still poorly understood section with ca. 50 species, which has aberrant members in Africa (e.g., *G. tomentosum* Thunb.) and Madagascar (e.g., *G.*

polyacanthum (Baker) Puff) and extends from Eurasia to North America. In Asia and Africa, *Galium* sect. *Trachygalium* is not clearly separable from the speciose *Galium* sect. *Galium*, including *Galium* sect. *Leiogalium* (≤ 100 or more species), which is centered in western Eurasia and the Mediterranean region. *Galium* sect. *Orientigalium* has its greatest diversity in southwestern Asia (> 50 species) and connects *Galium* sect. *Galium* with *Galium* sect. *Leptogalium* (ca. 42 species) in the western Mediterranean and Europe. For all these perennial sections, one should consult Ehrendorfer et al. (1976, 2005) and Chen and Ehrendorfer (2011). It is obvious that their taxonomic limits are somewhat vague, and none are clearly defined by available DNA sequences or by morphological criteria. Nevertheless, the data of Soza and Olmstead (2010a) and Ehrendorfer et al. (2011, and in prep.) give clear evidence on the independent and parallel origins of annual taxa from perennial sections of *Galium* s. str., particularly in the Mediterranean region and Southwest Asia. This refers to the annual sections *Galium* sect. *Aparine* (about eight species) and *Galium* sect. *Kolgyda* s. str. (three species), apparently derived from ancestors among the perennial *Galium* sect. *Hylaea*, as well as to the annual section *Galium* sect. *Microgalium* (ca. 15 species), closely related to perennials of *Galium* sect. *Leptogalium*.

General Conclusions

From our present understanding of the phylogenetic relationships of the Rubieae clades, it is obvious that today's taxonomic subdivisions within the tribe are still strongly influenced by tradition and should not be maintained. If taxonomy is expected to reflect evolutionary differentiation and affinities by descent, we need to revise the supraspecific taxonomy of Rubieae.

The first and obviously necessary step in this direction is the elimination of clearly polyphyletic taxa as *Asperula* s.l. Nevertheless, problems arise with paraphyly and from the cladistic postulate that only holophyletic taxa are acceptable, which include common ancestors as well as all of their descendants. If this postulate is violated, we will be accused of creating unacceptable paraphyletic taxa. Or should we interpret all extant ancestor-like plesiomorphic relics and descendant-like apomorphic expansive newcomers simply as phylogenetic sisters? Is this a legitimate way to eliminate many critical cases of paraphyly, or should we rather accept such paraphyletic supraspecific taxa? Thus, contrary to the arguments recently summarized by Schmidt-Lebuhn (2011) and in agreement with Hörandl (2006, 2007), Stuessy (2009), Hörandl and Stuessy (2010), and many others, we prefer the latter, less rigid, evolutionary, and also morphologically plausible approach: we accept paraphyly in DNA-supported cases, where extant, relatively plesiomorphic and rather unchanged ancestor-like taxa in their geological past appear to have given rise to strongly deviating and clearly more apomorphic descendant taxa (see Schmidt-Lebhuhn, 2011: fig. 2). Relevant examples from Rubieae discussed herein are that *Kelloggia* lies basal to all remaining Rubieae, followed by the well-supported distinction of *Didymaea* and *Rubia* from a remaining superclade of *Galium* s.l.; that *Galium* sect. *Cymogalia* is strongly supported as basal to the remaining species groups of *Galium* s.l.; that *Asperula* sect. *Cruciana* is suggested to be affined with *Crucianella*, *Phuopsis*, *Sherardia*, and the *Asperula* sections *Cynanchicae*, *Hexaphylla*, and *Thliphthisa*; that *Asperula* sect. *Glabella* is strongly supported as basal to *Galium* sect. *Aparinoides* and *Asperula* sect. *Dioicae*; that *Galium* sect. *Platygalium* appears to be derivative to *Relbunium*, *Bataprine*, and to many other species groups in the New World; that, furthermore, *Galium* sect. *Platygalium* is well supported in its affiliation with both *Cruciata* and *Valantia*; and that the perennial *Galium* sect. *Hylaea* can be postulated as basal to the annual *Galium* sect. *Aparine* and *Galium* sect. *Kolgyda* s. str., whereas *Galium* sect. *Leptogalium* apparently is derivative to the annual *Galium* sect. *Microgalium*.

Should one give preference to splitting or to lumping to improve the traditional Rubieae taxonomy? This question is not easy to answer, particularly with respect to the superclade of *Galium* s.l. (Fig. 2). In the case of splitting one could maintain the well-established and unproblematic genera *Asperula* s. str., *Callipeltis*, *Crucianella*, *Cruciata*, *Galium* s. str., *Mericarpaea*, *Phuopsis*, *Sherardia*, and *Valantia*. For the current *Asperula* sections—*Asperula* sect. *Cruciana*, *Asperula* sect. *Cynanchicae*, *Asperula* sect. *Glabella*, *Asperula* sect. *Hexaphylla*, and *Asperula* sect. *Thliphthisa*—and for the *Galium* sections—*Galium* sect. *Aparinoides*, *Galium* sect. *Cymogalia*, *Galium* sect. *Depauperata*, and *Galium* sect. *Platygalium*—one would have to create seven to nine new genera. If lumping is preferred, all of the aforementioned genera and sections evidently would have to be united and partly renamed under *Galium* s.l., together with the creation of a new and rather complex infrageneric taxonomical hierarchy to reflect affinities between the many subunits. Both approaches would be compatible with our present knowledge of phylogeny within Rubieae. Nevertheless, for practical reasons we strongly favor the splitting

alternative because it makes relationships within the speciose Rubieae better visible on the binomial level and can be achieved with fewer nomenclatural changes. We hope to approach this goal by a thoroughly documented and species-based phylogenetic analysis, which is in preparation.

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APPENDIX 1. List of taxonomic names of genera (in boldface) and sections within Rubieae (Rubiaceae) examined in the present study. *Bataprine* Nieuwl., *Microphysa* Schrenk, *Leptunis* Stev. (missing in the present molecular analysis), and *Relbunium* (Endl.) Benth. & Hook. f. are not accepted herein as genera. Apparently they have to be included in *Galium* sect. *Platygalium* s.l., with only *Leptunis* Stev. in *Asperula* sect. *Trichodes* Boiss., near section *Cruciana* Griseb.

Asperula L., Sp. Pl. 1: 103. 1753. *Asperula* L. sect. *Asperula*. TYPE: *Asperula arvensis* L.

Asperula L. sect. *Cruciana* Griseb., Spic. Fl. Rumel. 2: 166. 1846. TYPE: *Asperula glomerata* (M. Bieb.) Griseb.

Asperula L. sect. *Cynanchicae* (DC.) Boiss., Diagn. Pl. Orient, ser. 1, 3: 31. 1843. TYPE: *Asperula cynanchica* L.

Asperula L. sect. *Dioicae* Airy Shaw & Turrill, Bull. Misc. Inform. Kew 1928 (3): 82. 1928. LECTOTYPE: *Asperula conferta* Hook. f., designated by Ehrendorfer here.

Asperula L. sect. *Glabella* Griseb., Spic. Fl. Rumel. 2: 166. 1846. LECTOTYPE: *Asperula involucrata* Wahlenb., designated by Ehrendorfer, Bot. J. Linn. Soc. 70: 2, 1975.

Asperula L. sect. *Hexaphylla* Ehrend., Bot. J. Linn. Soc. 70(1): 2. 1975. TYPE: *Asperula hexaphylla* All.

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Bataprine Nieuwl., Amer. Midl. Naturalist 1: 264. 1910. TYPE: *Bataprine hispidula* (Michx.) Nieuwl. ≡ *Galium hispidulum* Michx.

Callipeltis Steven, Observ. Pl. Ross. (2): 69. 1828. [1824–Sep. 1828]); Nouv. Mém. Soc. Imp. Naturalistes Moscou 1: 275. 1829: TYPE: *Callipeltis cucullaris* (L.) DC., as "*cucullaria*" 1830, non *Callipeltis cucullaris* Steven, nom. inval., 1829.

Crucianella L., Sp. Pl. 1: 108. 1753. TYPE: *Crucianella latifolia* L.

Cruciata Mill., Gard. Dict. Abr. (ed. 4). 1754. *Galium* sect. *Cruciata* W. D. J. Koch, Syn. Fl. Germ. Helv. (ed. 2): 361. 1843. TYPE: *Cruciata laevipes* Opiz.

Didymaea Hook. f., Gen. Pl. [Bentham & Hooker f.] 2: 150. 1873. TYPE: *Didymaea mexicana* Hook. f.

Galium L., Sp. Pl. 1: 105. 1753. *Galium* L. sect. *Galium* [≡ *Galium* sect. *Eugalium* (DC.) W. D. J. Koch, Syn. Fl. Germ. Helv. (ed. 2): 364. 1843]. TYPE: *Galium verum* L.

Galium L. sect. *Aparine* W. D. J. Koch, Syn. Fl. Germ. Helv. (ed. 2): 362. 1843. TYPE: *Galium aparine* L.

Galium L. sect. *Aparinoides* (Jord.) Gren. Fl. France [Grenier] 2: 15, 39. 1851. TYPE: *Galium palustre* L.

Galium L. sect. *Cymogalia* Pobed. s. str., Fl. URSS 23: 712. 1958. TYPE: *Galium paradoxum* Maxim.

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Sherardia L., Sp. Pl. 1: 102. 1753.: *Sherardia arvensis* L.

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TAXONOMY VERSUS CLADONOMY IN THE DICOT FAMILIES[1]

R. K. Brummitt[2†]

Abstract

The theoretical basis for cladistic classification into monophyletic (holophyletic) ranked taxa is fatally flawed and is promoting bad taxonomy. Biological classification that takes account of evolutionary history may be based on two main factors—lines of descent and extent of divergence represented by morphological and other characters. In taxonomy a balance must be found between lines of descent and characters, and insistence on one at the expense of the other will give unacceptable results. Much confusion has arisen in systematics from the failure to appreciate that taxonomy, which groups organisms into ranked taxa (families, genera, etc.), is essentially different from grouping them into clades. These two processes are based on conflicting hierarchies and have different methodologies and functions. For several decades, however, the cladistics movement has adopted lines of descent rather than characters as the sole basis of taxonomy, insisting that only complete clades should be recognized as taxa. But as soon as one imposes ranks on a phylogeny, one must create paraphyletic taxa. These are natural products of evolution, which should be recognized in taxonomy. When ranks are adopted without acceptance of paraphyletic taxa, taxonomic free fall sets in, and every clade sinks into those taxa to which its original ancestor is referable. The clash of hierarchies results in absurdity, extending to sinking the entire plant kingdom into one family and one genus, but this has been strangely overlooked by the cladistic side. Adoption of ranked taxa is incompatible with recognition of only complete clades. Merely because one taxon falls phylogenetically within the clade of another taxon at the same rank does not necessarily mean that it must be included in it taxonomically. New characters will have arisen during evolution, which should be taken into account. A monophyletic (= holophyletic) system recognizing only complete clades is logically possible only if ranks are abandoned, as in the PhyloCode. It may be referred to as a "cladification" and the process producing it as "cladonomy," which are quite different concepts from a "classification" and "taxonomy." In a classification we have a hierarchical series of taxa at different ranks, while in a cladification we merely have a hierarchy of clades nesting within successively bigger clades. Cladistic taxonomy is particularly nonsensical in paleobotany, where our Linnaean taxonomic system and our code of nomenclature apply just as they do for extant plants. Cladograms are not classifications, and they need critical taxonomic assessment. The great majority of users of taxonomy are interested in characters and not cladistic theory. A general purpose classification is needed, requiring acceptance of paraphyletic taxa that are defined by characters as well as lines of descent. Examples in the dicot flowering plant families are given in which cladistic principles have imposed excessive insistence on lines of descent at the expense of evolution of characters, producing what many regard as bad taxonomy.

Key words: Cladification, cladistics, cladograms, cladonomy, classification, evolution, flowering plant families, hierarchies, paraphyly, taxonomy.

It has been over 150 years since Darwin published *On the Origin of Species*, and it will be surprising to some observers that in the 21st century there is still heated disagreement on how the theory of evolution affects the classification of plants and animals. I entered the fray of this argument the best part of 20 years ago and have somehow survived all the slings and arrows that have been sent my way since. I said some years ago that this is the most important issue under debate in biological systematics today, and I feel that nobody has ever seriously challenged the critical views on cladistics that I and many other taxonomists have put forward. Open discussion is needed to resolve this persistent aggravating problem.

Two Different Processes

There is a major schism between two schools of thought on how we should derive a classification from the evolutionary history of a group. Those persuaded by the cladistics movement emphasize past lines of descent as the only basis for classification. They insist that all taxa must be monophyletic, that is, they should be complete clades, so one family cannot have evolved from within another family because that would be an incomplete clade and so paraphyletic. Their units are then defined by lines of descent (ancestry) rather than by characters. The result is a "cladification," and in my title I have called the process "cladonomy," having adopted these terms 15 years ago (Brummitt, 1997) from the late and great

[1] Based on the text (with some additions) of a video presentation given to the XVIII International Botanical Congress in Melbourne in July 2011.

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zoologist Ernst Mayr. The other school allows that *characters* are important and have changed through evolution, so that one taxon may have *evolved* from within others at the same and presumably lower ranks. This enables us to produce a "classification," taking into account evolution of characters, as distinct from a "cladification" based only on lines of descent.

"Taxonomy" is the naming of taxa while "cladonomy" is the naming of clades, and "cladistic taxonomy" is an oxymoron (Knox, 1998: 5). In recent decades the process of recognizing groups according to their descent *and* their characters has become known as "evolutionary taxonomy," a very appropriate term also popularized by Ernst Mayr (see Mayr & Ashlock, 1991, and references therein, and a historical review by Ashlock, 1974). Stuessy (2006) has noted that the term "phyletics" also takes into account the degree of divergence, whereas "cladistics" uses only the branching pattern. Hörandl (2006) has emphasized the difference between a classification based on evolution of characters and one based on cladistic theory, and she titled her article, "Neglecting Evolution Is Bad Taxonomy" (Hörandl, 2007). Cladistics indeed defines taxa solely by their ancestry and ignores the evolution of characters (Stuessy & König, 2009). In broad reviews of the subject, Stuessy (2009) and Hörandl and Stuessy (2010) have, like other authors, stressed that paraphyletic taxa defined by descent *and* characters are natural units in evolution and are not only desirable but indeed inevitable in taxonomy.

Basic Concepts Confused

So how is it that these two schools of thought have developed alongside each other? Following the publication of Hennig's influential ideas on cladistic analysis (1966), the view was widely propagated that this must be the basis of modern taxonomy. Many philosophical papers of the 1960s to 1990s were concerned with definitions and how Hennig's methods could recreate actual phylogenetic history in terms of clades—see, for example, Nelson (1971), Farris (1974), Platnick (1979), or Patterson (1982). All commentators on the cladistic side apparently made a fatal assumption that there is only one way to chop up a phylogenetic tree, and they concluded that all taxa must be monophyletic—that is, complete clades. But they did not address the question of how to produce a classification of clades into ranked taxa without any being paraphyletic. In fact, ranks were never even mentioned. So application of the formal taxonomic hierarchy to a phylogeny was not considered at all. Although this is fundamental to taxonomy, it has been completely ignored in a large body of theoretical literature on cladistics up to the present day. But as soon as one applies ranks to a phylogenetic tree in order to produce a classification, one must create paraphyletic taxa. Every suprageneric taxon accepted has apparently descended from something recognizable at around species level and must make another taxon paraphyletic. If paraphyletic taxa are not accepted, every clade (including the entire plant kingdom) will sink into those taxa (family, genus, perhaps species) to which their ancestor is referred. The phylogenetic tree can thus be chopped up according to evolution of characters, creating a taxonomy, in a quite different way from recognizing only complete clades. Evolution of ranked taxa is paraphyly all the way. The hierarchy of descent is not the same as the taxonomic hierarchy. It was left to De Queiroz and Gauthier (1992) to state the obvious conclusion that cladistic classification is incompatible with ranked taxa. It is a simple point, but failure to recognize the difference between taxonomy and cladonomy is the cause of many of our current problems in systematics.

But as new methods of data analysis developed driven by computer programs, the new thinking of cladistics became fashionable and was widely but uncritically accepted by many as the correct and only way forward for taxonomy. It was argued that lines of descent are objective matters of historical fact, and, even though they are impossible to observe directly, this must take precedence over use of *characters* as formerly adopted as the basis of taxonomy. It is true that traditional classification has an element of subjectivity in defining taxa according to characters, but at least it can be based on direct observation. But all classification is subjective in assignation of ranks to taxa, and evolutionary taxonomy is no more subjective than is "cladistic taxonomy." In cladistic thinking, all taxa must be monophyletic (= holophyletic, i.e., complete clades), and the view that paraphyletic taxa (incomplete clades reflecting evolution of characters) are anathema was widely—but not universally—accepted.

It is fundamental to the present discussion that taxonomy and cladonomy are quite different processes with different functions. Taxa are grades showing maximum correlation of characters and are essentially different from clades, and the two concepts cannot be combined in one system. They are logically incompatible with each other, though they may often coincide since many recently evolved taxa are also clades. The differences between grades and clades have long been apparent in discussions of taxonomic theory where these contrasting concepts have been recognized

(Davis & Heywood, 1963; Cronquist, 1987: 2, 8–9). The Venn diagram of Brummitt (1996: fig. 1, and 2002: fig. 2) illustrates how the hierarchies of phylogeny and of classification conflict with each other and cannot be equated. In practice, we need to choose which we want according to the use to which it will be put. Ashlock (1974) has demonstrated that in phytogeographical analysis we need to adopt monophyletic groups (clades), but in creating a taxonomy this is inappropriate, and we need taxa, which inevitably create paraphyly. However, this fundamental difference was overlooked by many commentators in the cladistic scene. A quite different criterion for classification was thus introduced with very little consideration of its logical basis or the practical effects it would have. The mood of many was for a new thinking stressing ancestry at the expense of evolution of characters, but this is now seriously challenged.

The Present Argument

We now have evolutionary taxonomy competing with "cladistic taxonomy," each with deeply entrenched lines of supporters. It is not just a matter of one way of operating being rather better than the other. It is, in my opinion, a case of one theory or principle being clearly logically **wrong** and the other logically **correct.** Evolution is an ongoing process of change through time. To divide a phylogenetic tree representing development of taxa over a period of time into families, genera, and species without any of them being descended from within another taxon at the same or lower rank is a logical absurdity. It should be obvious that, if this were accepted, no new taxa could ever have evolved. Every taxon would sink into another. No genera or families or higher taxa could be recognized, and the entire plant kingdom would sink into one family and genus. I have here referred to this phenomenon as "taxonomic free fall," having previously referred to it as "infinite regression" (Brummitt, 1996: 376). Our theory of classification will be incompatible with evolution if it cannot accommodate all the products of the evolutionary process, including fossils and other extinct plants. And if it ignores characters, it will be nonsensical to users. If it does not accept paraphyletic taxa, which are natural products of evolution defined by characters, it is surely not acceptable. **The theoretical basis for cladistic classification into monophyletic ranked taxa is fatally flawed and is promoting bad taxonomy.**

The cladistic view would require that as evolution produces wider and wider variation, the rank of the taxa that result has to be progressively lower and lower (see examples quoted below, particularly Cactaceae). How can anyone believe this? To me, it is **nonsensical**. Common sense would lead us to expect the opposite. The taxonomic hierarchy and the hierarchy of descent, which work in opposite directions, have been confused. It has been said with disarming honesty on the cladistic side (Albach et al., 2004: 429) that, if you want ranks *and* only monophyletic taxa, you must either sink taxa that are quite distinct (see many examples below, particularly Scyphostegiaceae and Lennoaceae) or split taxa where there are no appropriate characters to do so (see Portulacaceae and Scrophulariaceae below). This means that characters are ignored and taxonomy becomes nonsensical to users. There *must* be something fundamentally wrong with this. Characters are essential to taxonomy, but they are neglected in cladistic classification.

Cronquist (1987: 2) was among the first to appreciate the problems of cladistic taxonomy, pointing out that the refusal to accept paraphyletic taxa is a fatal flaw in the theory behind it. He noted that such taxonomy assigns ranks to organisms "entirely on the basis of recency of common descent" while "the *amount* of divergence scarcely enters the picture." At an Association for the Taxonomic Study of the Flora of Tropical Africa (AETFAT) Congress in 1994, the logical impossibility of combining monophyly with ranked taxa was emphasized again (Brummitt, 1996; but see also Schrire & Lewis, 1996). Some 15 years ago, Sosef (1997) referred to the inevitability of supraspecific paraphyletic taxa. In the same year Stuessy (1997) pointed out that classification should be based on more than just branching patterns. Over the last quarter of a century it has been increasingly argued—often from different perspectives—that recognition of taxa based on characters is a quite different process from recognition of clades, and they should not be confused (Buck, 1986; Cronquist, 1987; Hedberg, 1995; Brummitt, 1996, 1997, 2002, 2008; Sosef, 1997; Stuessy, 1997, 2006, 2009; van Welzen, 1997; Brummitt & Sosef, 1998; Knox, 1998; Diggs & Lipscomb, 2002; Grant, 2003; Nordal & Stedje, 2005; Stace, 2005; Cullen & Walters, 2006; Hörandl, 2006, 2007, 2010; Farjon, 2007, 2009; van Wyk, 2007; Zander, 2007; Cullen in Brickell et al., 2008; Stuessy & König, 2009; Hörandl & Stuessy, 2010; Podani, 2010).

But those who are seduced by the superficial attractiveness of a system of ranked taxa that are all complete clades (i.e., containing only holophyletic groups) have been unwilling to accept that this is a logical impossibility, or they have chosen to ignore it. So the taxonomic community is now divided on a major principle. It has been said that paraphyletic

groups cannot be recognized as taxa because they have no real biological existence, but what is meant is merely that they are not complete clades, which is self-evident from their definition. Such groups are the unavoidable consequence of anagenesis in evolution, and they exist as real biological entities through their definition by characters (Hörandl & Stuessy, 2010). Claims from the cladistic side that paraphyletic groups have no defining characters (Patterson, 1982) seem strange. Such groups are just as much a reflection of how evolution has progressed as is the concept of monophyly and should be accepted in our taxonomy. Ignoring evolution of new characters is bad taxonomy (again, Hörandl, 2007).

So What *Should* We Do?

If you insist on only monophyletic (= holophyletic) units (clades), you have to abandon ranks, and then the concept of paraphyletic taxa cannot apply. You then just have clades nesting within bigger and bigger clades. This was realized by proponents of the PhyloCode two decades ago, and De Queiroz and Gauthier (1994), in their second paper, proposed a new taxonomy with new nomenclature that abandoned ranks (see also Moore, 1998; De Queiroz & Cantino, 2001; Cantino et al., 2007). There would be no families, no genera, no binomials for species—only clades. But that is not practical taxonomy, and it has not gained general acceptance in the botanical world. The PhyloCode was logical (a good example of cladonomy), even if not practical, but cladistic taxonomy is neither logical nor practical. Those who want their classification to recognize only clades irrespective of characters are asking for something very narrowly specialized (Cronquist, 1987: 2; Stace, 2005). They should adopt the appropriate approach and abandon ranks instead of trying to replace the taxon-based classification that most users need.

In recent decades those wanting only monophyletic taxa have been boosted by the revolution in molecular systematics, and those like me on the other side of the cladistic argument have been castigated as being out of touch with modern developments. But it is not the evolutionary taxonomists who are behind in their ideas; it is the cladistic side, who have failed to respond to the logical arguments that have been advanced by their opponents. Now the tide has turned, and the thinking has moved on since the late 20th century. I am a great supporter of molecular data—so long as they are used properly. But the cladistic arguments that usually accompany molecular analyses are not driving taxonomy forward; they are putting it into reverse. As van Wyk (2007: 647) has said, "It is ironic that at a time when phylogenetic understanding is at an all time best, the usefulness of classifications has deteriorated to the point of becoming absurd." Indeed, the nearer that modern technologies bring us to reconstructing past evolution, the greater is the absurdity of cladistic classification. If we knew everything, we would have only one family and one genus.

For those who think that traditional taxonomy is just too old-fashioned to be recognized as serious science, I would note that in 1997 a formal debate at the Linnean Society of London voted heavily against cladistic theory and in favor of evolutionary taxonomy (see Brummitt, 2002: 40). In the last decade, some 170 practicing taxonomists, including many senior figures, have put their names to statements in *Taxon* opposing the cladistic line (see especially Nordal & Stedje, 2005). Most users of our taxonomy are not interested in cladistic theory; they are interested in the characters of the taxa we recognize (Cullen in Brickell et al., 2008). The practical importance of taxonomy has similarly been stressed by Diggs and Lipscomb (2002), Stace (2005), van Wyk (2007), and others. Grant (2003) has emphasized the incongruence of cladistic and taxonomic systems. Cladistic treatments of well-known groups have been considered unacceptable (Farjon, 2009). Recent arguments that have been advanced favoring the cladistic view (Nelson et al., 2003; Wiley, 2009) have been refuted (Brummitt, 2003; Stuessy & König, 2009). In a perceptive historical overview of family classification, Cullen and Walters (2006) have said that the principle of monophyly (holophyly) is now discredited and of zero value.

Similar problems are under debate in zoology, as seen in the difference of opinion over the sinking of the leeches into the earthworms (Schmelz & Timm, 2008). And even a botanist can reflect that mammals nest phylogenetically within bony fishes and so should be sunk according to cladistic theory (Brummitt, 2006). The logical and practical arguments against cladistic taxonomy at the very least demand serious consideration, but they are ignored by many, and bad taxonomy continues to pour forth.

Phylogenies versus Cladograms

One point of view that is probably widely held on the cladistic side has been well expressed by Potter and Freudenstein (2005) and requires comment (see also Freudenstein, 1998; and response by Brummitt & Sosef, 1998). They have argued for both monophyly and ranked taxa in the same system and have rightly drawn attention to essential differences between phylogenetic trees and cladograms. Because we do not have the extinct ancestors and so cannot know the

"true underlying phylogeny" (Potter & Freudenstein, 2005: 1034), they prefer to base their classification on cladograms rather than phylogenetic trees. But cladograms do not include ancestors or a timescale, which are essential to the concepts of holophyly and paraphyly, and they do not determine the ranks of their various branches. They commonly represent taxa only at a single point in time—a synchronous classification in the terminology of Podani (2009, 2010)—since, with rare exceptions, only present-day taxa are included. A diachronous classification, by contrast, includes all extinct and living organisms, and a phylogenetic tree remains the only meaningful representation of ancestor/descendant relationships (Podani, 2010). Dealing only with cladograms means that change of characters over time, and consequently evolution of new taxa, are not revealed.

The view is sometimes put forward that, if we are dealing only with extant plants and will accept a synchronous classification, it is possible to recognize only holophyletic taxa. But this only emphasizes that cladistic theory is incompatible with the ongoing process of evolution through time. If we were concerned only with extant plants and discounting their ancestors, nothing would be paraphyletic since present-day plants have not evolved from other present-day plants. On the other hand, if cladistics postulates extinct ancestors in order to demonstrate paraphyly, then it must also postulate extinct ancestors that produce taxonomic free fall, causing all classifications to collapse into one taxon. Just because we cannot name an ancestor does not mean that ancestors did not exist or that they do not need to be considered. We should not confuse taxonomy with nomenclature. Cladograms cannot assess taxonomic significance of the development of new characters (see discussion of Scyphostegiaceae below). Podani (2010) has considered that paraphyly is a mathematical necessity in diachronous phylogenetic classification. When we consider that our principles of Linnaean classification and our code of nomenclature apply to paleobotany as well as to extant taxa, the cladistic argument is manifestly untenable.

Cladograms are not immutable since their topology may change with the inclusion of more or fewer taxa or different characters in the analysis, and indeed the same data set may give multiple cladograms. Furthermore, it is doubtful whether cladistic analysis of only extant taxa ever gives an accurate picture of past evolution as directly evidenced by fossils (Axsmith et al., 1998; Farjon, 2007; see also Hedberg, 1996). If we could build extinct plants into our cladograms that are based on extant plants, the topology would very probably change considerably. Consider, for example, what we know (or, more important, what we don't know) about the evolution of Hydrostachyaceae—see below. Should we really ignore the taxonomic importance of *characters* and derive our classification entirely from topology of diagrams that are based only on present-day taxa and probably do not accurately reflect the true underlying phylogeny (Grant, 2001; see also splitting of Flacourtiaceae, below)? Characters may, of course, be used in the production of a cladogram and may be highlighted within it, but according to cladistic theory they do not play any part in taxonomic assessment. Cladograms give useful visual impressions of phylogenetic relationships, but they do not determine ranks and are not classifications (Brummitt, 1996). They need critical taxonomic interpretation. As Stuessy (1997) and Stuessy and König (2009) have argued convincingly, we need to look beyond the topology of cladograms in producing a classification. See also Ashlock (1974: 94–97).

Phylogenetic trees, on the other hand, include a timescale and ancestors and are a better representation of actual phylogeny. But the fundamental question is not whether we should base our classification on phylogenetic trees or on cladograms. The arguments are just the same either way. The basic question is whether we can accept that one family can have evolved from within another family, one genus from within another genus, and so on. Why should that not be so? To those who believe in evolution, it would seem to be the inevitable assumption. Where else did they come from? As soon as you apply ranks to a phylogenetic tree, you must create paraphyletic taxa. **So why should we try to make all taxa holophyletic in our taxonomy?** It makes no sense. Have we had development from unicellular algae to advanced angiosperms without anything ever giving rise to a new group recognizable at the same or higher rank? The whole theory of cladistic taxonomy is in conflict with the evolution of characters and new taxa and defies natural intuition. Whether we work with phylogenies or cladograms, so-called cladistic taxonomy is unacceptable because it is incompatible with development of new taxa and with Linnaean nomenclature.

Examples in Dicot Families[1]

All these considerations affect family concepts in the dicots. Please note that I am not talking about a

[1] Names of families and other taxa affected by the preceding discussion are here printed in boldface for ease of reference. This does not reflect any particular taxonomic conclusion.

linear sequence of families (where I am largely supportive of the latest conclusions based on molecular data); I am talking only about how the families are defined. I know nothing worth knowing about the monocots but have worked my way through the dicot families over the last 15 years. When I compared my list of dicot families with those recognized by authors accepting cladistic principles,[2] I found that there were 112 differences out of a total of about 400 families—about 25% to 30% disagreement. Not all of these were the direct result of paraphyly, since some were due merely to the associated problem of failure to take characters into account. Most are cases where cladists sink families where I would split, but in a few instances it is the other way round. I have selected a few cases to show how paraphyly or failure to recognize characters can be the major factor.

I am starting with the pantropical and Southern Hemisphere traditional family **Flacourtiaceae**. First, let me say that I have agreed that *Aphloia* (DC.) Benn. and *Berberidopsis* Hook. f. have to be taken out of Flacourtiaceae because both the molecular data and the morphological evidence support it. But then we come to *Scyphostegia* Stapf, which has long been treated as a monotypic family **Scyphostegiaceae** endemic to Borneo. It is indeed a very strange plant in its reproductive structures. The male inflorescence is compact, oblong to linear, up to 5 × 2 cm, consisting of 20 to 25 very short concentric tubular bracts, like a mini Leaning Tower of Pisa. Nestling within these short tubes are the inconspicuous male flowers, one per bract, with a green tubular perianth with three long and three short lobes, and three stamens united into a column. The female inflorescence has usually only two short concentric bracts and again a shortly tubular perianth, and the ovary has many erect elongate ovules crowded together on its floor. I wonder if you have been counting the number of important features that do not characterize traditional Flacourtiaceae—unique extraordinary inflorescences, fused perianth (as distinct from free sepals and petals), six unequal perianth lobes, only three stamens (as distinct from usually very many), a staminal column, many basal (not parietal) ovules, and so on. In discussing various suggested possible affinities of *Scyphostegia*, van Steenis (1972) commented that all recent authors agreed that it is a distinct family on its own. But cladonomy ignores characters and is based on quite different criteria from taxonomy, and cladists sink Scyphostegiaceae into the traditional concept of Flacourtiaceae. It does not matter to them how many differences there are or how conspicuous these are. If it falls within another family in their cladogram, they are very sorry—they will say—but they have to sink it. To them, characters are irrelevant. Everything depends on presumed lines of descent. You must ignore the evolution of new characters. But, again as Hörandl (2007) has said, neglecting evolution is bad taxonomy.

But there is more. The cladogram based on *rbcL* sequences presented in Chase et al. (2002) divides the 80 or so genera of traditional Flacourtiaceae into two major groups that are now treated in the cladistic literature as separate families. But only 30 out of the 80 genera were sampled, including only one species of the basal tribe Casearieae, which has 13 genera (including *Casearia* Jacq. with some 180 species). The characters quoted in the paper show no morphological support for recognizing two families, and experienced taxonomists who have worked extensively in this group tell me they see no good case for splitting the traditional family in this way. Relying solely on the topology of this cladogram does not seem to me to be necessarily good taxonomy. One wonders whether more complete sampling at the base of the tree might not hold the traditional family together.

Furthermore, in one part of the cladogram is a South African group of three monotypic genera long known as the family **Achariaceae** (see van Wyk, 1988), nesting within one half of the whole group and so sunk to tribal level. They differ markedly from traditional Flacourtiaceae in their habit (herbaceous rosettes or climbers with palmate leaves, rather than trees and shrubs), their exstipulate leaves, a strongly *gamopetalous* corolla rather than free petals, and only three to five stamens rather than usually very many. But again, despite the presence of these striking characters, cladistic theory has sunk this family, and many will consider that to be a retrograde step. Evolutionary taxonomy would allow us to assess *characters* and take our own decisions, but cladistics ignores characters and always rigidly forces us into one position, which many taxonomists would reject as being simply bad taxonomy.

But worse is to come. It turns out that the name "Achariaceae" is the earliest family name in the group, so the cladistically motivated are now obliged

[2] Cladistic views referred to here are those employed in the third system published by the Angiosperm Phylogeny Group (2009), popularly known as APG III, and Mabberley (2008), unless a different reference is given. In some cases alternative conclusions are reached by other authors. Other references to taxonomic papers are cited in the text. Where no citation is given, a useful source for many further references to the taxonomy of families mentioned, plus many illustrations, with examples and discussions of the classifications preferred here, can be found in Heywood et al. (2007).

to use that name for half the traditional Flacourts while those not so constrained keep it for the three South African genera. But when Achariaceae are demoted to become the tribe **Acharieae**, they nest within the tribe Pangieae, so that is just as paraphyletic as the broad family was. Sinking the family just transfers paraphyly to a different rank. The distinctive group long recognized as a family by evolutionary taxonomists must have a rank *lower than tribe* in cladistic classification. In the absence of the restraining effect that consideration of characters would afford, taxonomic free fall is setting in. This sort of thing is always liable to happen when we start sinking on cladistic principles, and if we had access to the ancestors, all 80 genera of traditional Flacourtiaceae would sink into one genus. The theory is all wrong. But when families are sunk on cladistic principles, the logical infrafamilial classification into monophyletic taxa is seldom considered. If you want a monophyletic system, you can have your wish, but only if you abolish ranks. It seems to me—and to many others—that cladistic classification into ranked taxa is both illogical in theory and disastrous in practice!

But there is still more bad news. Also in the cladogram we have the traditional **Salicaceae** (*Salix* L. and *Populus* L. with catkinate inflorescences, apetalous flowers, usually only two stamens in *Salix* and five in *Populus* against usually very many in traditional Flacourtiaceae, and distinctive fruits and seeds) nesting within the other major group of traditional Flacourts. So cladists again argue that it is essential to sink the family, and they call them tribe Saliceae. But the new tribe **Saliceae** nests within the tribe Flacourtieae, which is then just as paraphyletic as the family was. Again, sinking the family does not avoid paraphyly, and the traditional family Salicaceae must also sink to *below tribal level*. We have taxonomic free fall again. Characters are ignored. And again this is accompanied by a nomenclatural upheaval since in cladistic thinking the other half of the old Flacourtiaceae now has to be called Salicaceae, causing widespread confusion, and the name Flacourtiaceae falls into its synonymy. The name Salicaceae is now in use in two widely different senses. Many taxonomists would prefer to keep Flacourtiaceae in its traditional sense with three families apparently having evolved separately from within it, so we have a polyparaphyletic family. None of the three epiphyletic families had ever been sunk into Flacourtiaceae before on morphological grounds.

My next example is a group of families that used to constitute the order Primulales. Usually I don't query the mechanism for producing the cladogram, but when I find it is claimed that the arborescent tropical Myrsinaceae have evolved from the largely temperate herbaceous **Primulaceae** (Anderberg et al., 1998: 99), this seems to be the reverse of intuitive expectation, and I wonder if somebody should test the analysis again. Cladists, of course, recognized clades instead of taxa, and they at first combined one half of Primulaceae with Myrsinaceae, placing genera such as *Cyclamen* L. in Myrsinaceae (see Ståhl & Anderberg, 2004). Extraordinary indeed, but exactly the sort of thing that you might expect if only clades are recognized and characters are ignored. Later, the Angiosperm Phylogeny Group (2009) went a lot further and restored *Cyclamen* to its natural family by sinking the perfectly acceptable families **Myrsinaceae**, **Maesaceae**, **Theophrastaceae**, **Samolaceae**, and **Coridaceae** all into Primulaceae. This is typical of the excessive lumping that results when the holy grail of holophyly catches the imagination and characters are ignored. Again, no infrafamilial classification into only monophyletic taxa has been offered. And I must also observe that according to cladistic principles, 11 of the 23 genera of traditional Primulaceae should be sunk into other genera—six (including *Dodecatheon* L., *Soldanella* L., and the usually caespitose *Dionysia* Fenzl) into *Primula* L. (Mast et al., 2004), three into *Androsace* L., and two into *Lysimachia* L. I am getting that sinking feeling again. You can have a monophyletic system if you abandon ranks and ignore characters to produce a cladification, but in a taxonomic classification we need characters and ranks.

Another group that has suffered recently are Boraginales, now sunk by cladists into one family **Boraginaceae**. Within this is a very distinctive traditional family, **Lennoaceae**, with four parasitic species in two genera from California to Venezuela recognized in the revision by Yatskievych and Mason (1986). Parasitism alone does not necessarily make it a good family, but the 5- to 9-merous flowers, and the ovary of five to nine (to 16) carpels producing a fruit dehiscing by a circumscissile lid to reveal a pie chart of up to 28 wedge-shaped seeds cannot be accommodated in my concept of Boraginaceae. Scarcely less extraordinary is the sinking into Boraginaceae of **Hoplestigmataceae**, which has two western African tree species with a calyx of two to five irregular segments, nine to 14 corolla lobes, 20 to 25 stamens, and a drupe $\leq$ 3 cm long with a leathery exocarp and bony endocarp. Boraginaceae? No way! Also sunk are **Hydrophyllaceae**, with 15 genera and 300 species confined to the New World, which has virtually always been recognized as a separate family, and the African **Wellstediaceae**, with seven species in one

genus, which has also often been recognized. Both differ significantly from traditional Boraginaceae in having dehiscent capsules instead of four nutlets or fleshy pyrenes, and there is no need to sink these established families if you just accept the importance of characters and recognize paraphyletic taxa. Today I also recognize a family **Codonaceae** for one genus *Codon* L. (type) with two annual species from South Africa. It was recently rightly removed on morphological grounds from Hydrophyllaceae by Retief and van Wyk (2005), who made it a new subfamily Codonoideae in Boraginaceae (2005: 79), but it is very different from that family in its 10- to 12-merous (rather than 4- to 5-merous) flowers, many-seeded capsules (instead of four nutlets or pyrenes), both spinelike and glandular trichomes (unlike Boraginaceae), pollen with Ubisch bodies (as in Wellstediaceae), and other characters. Characters do matter in taxonomy.

If our taxonomy is too far removed from the intuitive appreciation of its users, they will just not accept it. An outstanding example is the sinking of **Valerianaceae**, **Dipsacaceae**, **Morinaceae**, and **Triplostegiaceae** into paraphyletic traditional **Caprifoliaceae**, but at least this is more inclined to provoke mere mirth rather than stringent criticism. It is sufficient to say that characters have again been ignored. The sinking into Malvaceae of nine families that have been recognized by Cheek (2007) in *Flowering Plant Families of the World*—**Sterculiaceae**, Pentapetaceae (now **Dombeyaceae** nom. conserv.), **Bombacaceae**, **Tiliaceae**, **Sparrmanniaceae**, **Brownlowiaceae**, **Byttneriaceae**, **Helicteraceae**, and **Durionaceae**—may not be an obvious case of paraphyly, but it is again the result of disregard of characters producing excessively broad taxa (Bayer et al., 1999). The lime tree, baobab, and durian do not sit comfortably in Malvaceae. If you want an Australian example, I could argue that it is not compulsory to sink herbaceous Tremandraceae into paraphyletic arborescent traditional **Elaeocarpaceae**, when the former has virtually none of the characters regularly quoted to characterize the latter.

The aquatic habitat is presumed to have provoked rapid evolution in many plants and so is likely to have produced good examples of paraphyletic taxa. The extraordinary aquatic family **Hydrostachyaceae**, one genus of 22 species widespread in fast-flowing water in the Old World tropics, is so weird that I cannot even work out how roots, stems, and leaves are to be distinguished. It is found in several different molecular analyses (see Albach et al., 2001) to nest with two New World genera of shrubs of **Hydrangeaceae** from the semidesert regions of the southwestern United States. But instead of sinking the family into Hydrangeaceae, and then logically into its tribe Philadelphieae (taxonomic free fall setting in again), those interested are now trying to find technical reasons to discount the several molecular analyses that put it there. It seems there are situations in which those adopting cladistic principles find the results too embarrassing to actually implement their conclusions.

A similar case concerns another family of fast-moving water, the **Podostemaceae**, where the habitat has again imposed a somewhat extreme morphological structure. It is agreed, perhaps surprisingly, that the family comes phylogenetically next to *Hypericum* L. (e.g., Davis & Chase, 2004), which most evolutionary taxonomists would place in **Clusiaceae**. But cladists involved decline to sink the nearly 50 genera of Podostemaceae into Clusiaceae, arguing instead that it is easier to recognize Hypericaceae (which few evolutionary taxonomists would agree with) and assume the Podostems will conveniently fall between that and Clusiaceae. This seems to me to be an unwarranted simplistic assumption relying excessively on cladogram topology and ignoring characters. Why can we not simply accept the family Podostemaceae (which has very many more distinctive characters than Hypericaceae would have) as evolutionary taxonomists have always done? The status of one taxon should not dictate the status of another. Podostemaceae did not arise from nothing by special creation; it evolved from within a different family, presumably Clusiaceae, which must be paraphyletic. No problem. Then *Hypericum* can stay in Clusiaceae.

Another case where it seems to be too much for cladists to actually accept their own principles is the potential sinking of **Cactaceae**—already made up of four subfamilies—into paraphyletic traditional **Portulacaceae**. Then logically, following taxonomic free fall again, it should sink to *below tribal level* in whichever tribe includes *Talinum* (classifications of the family vary; see Hershkovitz & Zimmer, 1997). Do we really need to sink four subfamilies together at a level lower than tribe? Again, the theory behind it is nonsensical. But Cactaceae is a high-profile family, and sinking it might make cladistic taxonomists look silly and cost them support among the general public. So they don't do it. Similarly a parallel succulent family in Madagascar and Africa, **Didiereaceae**, now divided into three subfamilies (Applequist & Wallace, 2003), also nests within the traditional Portulacaceae and on cladistic principles should also be sunk to *below tribal level* in that family. The alternative cladistic strategy, noted above, of splitting

the paraphyletic group rather than sinking another taxon into it, has resulted in the recent (Nyffeler & Eggli, 2010) dismemberment of the traditional Portulacaceae into several very narrowly defined families including **Montiaceae**, **Talinaceae**, and **Anacampserotaceae**, which does not seem helpful or desirable.

An extreme case of splitting concerns **Scrophulariaceae**. A first molecular analysis (based, it must be said, on the very limited sampling of 38 genera in a family of ca. 300) suggested that it should be split up into several clades that were to be treated as families (Olmstead et al., 2001), even though there were no characters quoted to justify this. More segregate families have been split off since, but I have not seen anybody try to key them out using *characters*. This seems to be a reversal of the principle behind the sinking of nine families into Malvaceae, referred to above. Why should we split in one case and sink in the other? Again, the importance of characters is ignored. And then other families where there do seem to be good characters, such as **Plantaginaceae**, **Hippuridaceae**, and **Callitrichaceae**, have had to be sunk on cladistic theory into Scroph segregates. And as in Flacourtiaceae, discussed above, awful nomenclatural upheavals follow. The name Plantaginaceae is still applied by many to a well-known family of three genera with very distinctive ovary structures, but it is now applied in cladistic literature to nearly 100 genera formerly referred to Scrophulariaceae. And at generic level, both *Bougueria* Decne. (producing a nut with a single peltate seed) and *Littorella* P. J. Bergius have been sunk into *Plantago* L. on cladistic principles (Schwarzbach, 2004). That might be good cladonomy if ranks were abandoned, but it is bad taxonomy. This sort of thing will always happen when completely different taxonomic principles are confused, and it is very disruptive.

Finally, perhaps we should raise our sights above the family level. For two centuries the flowering plants have been instinctively divided into dicots and monocots. Today it is not really doubted that the monocots are a monophyletic group, but it is established by molecular and other data that they have split off from dicot ancestors, leaving the **dicots** as a paraphyletic group. So our intuitive grouping of the flowering plants has been abandoned by the cladists, and the dicots are not recognized as a group distinct from the monocots. Are we not losing some useful taxonomy in all this? But if we have all the relevant information, we must realize that if the monocots are monophyletic, they must have arisen from within one *family* of dicots and so should be sunk completely into that. That is the logical conclusion of cladistic classification. We have taxonomic free fall waiting to happen again. And since the Rosids, Asterids, and Eudicots must have evolved from something, presumably at least the **Magnoliids** are also paraphyletic. We cannot escape paraphyly. See also Stuessy (2010).

Conclusions

While the methodology of cladistic analysis has facilitated welcome new perspectives in taxonomy, and major advances in reconstructing phylogeny have been made recently by molecular methods, in our taxonomy we must resist the temptation to overemphasize these developments. There are many more examples I could give where cladistics has upset evolutionary family classification. Cases at generic rank are perhaps even more aggravating to the general public, and the controversial sinking of *Dryandra* R. Br. to a mere series of paraphyletic *Banksia* L. f. (taxonomic free fall again) is a current talking point here in Australia (see George, 2014, this issue). In my opinion cladistic classification into ranked taxa without any being paraphyletic has been the worst fad ever to hit taxonomy. The whole theory behind it is nonsensical, based on the logical impossibility of recognizing only complete clades as taxa and yet applying ranks to them. As a result, alas, characters are ignored, and much bad taxonomy is produced today. Whenever a modern revision claims that all taxa are now monophyletic (= holophyletic), suspicions are immediately aroused about the new taxonomy.

The impressive review of family classification by Cullen and Walters (2006) has raised the question of whether we can ever hope for a broadly agreed family list. They have emphasized that recent systems, though rightly taking account of new information from modern technologies, are not always the optimal result for users of taxonomy. They have tentatively suggested that a body could be set up, including both producers and users of taxonomy, to recommend an agreed list of families and to review this at alternate International Botanical Congresses (i.e., every 12 years). In principle this is an attractive idea, but at present it would be extremely difficult to achieve anything near general agreement. Nonetheless, it is worth thinking about. The question of monophyly versus characters should no doubt be high on the agenda. The taxonomic community has an obligation to respond to the needs of the users.

Cladistics disregards the importance of characters, fails to recognize newly evolved taxa, does not represent evolutionary reality, is increasingly disruptive of orthodox taxonomy, conflicts with natural

intuition, does not provide users with information they require, is ultimately a logical impossibility, and, above all, tends to produce bad taxonomy. But as this symposium on paraphyly shows, cladistic taxonomy with its illogical obsession with holophyly is now increasingly being challenged. Can we not ease cladistic taxonomy comfortably to a quiet resting place in biological history? Common sense and good taxonomy must inevitably return in due course.

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SUPPORT MEASURES FOR CAULISTIC MACROEVOLUTIONARY TRANSFORMATIONS IN EVOLUTIONARY TREES[1]

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ABSTRACT

Investigation of caulistic macroevolution in an evolutionary tree often requires separate support measures for exemplar groups, and for the taxon they represent. A taxon may be actually or cryptically heterophyletic on a molecular tree. Congruence between branch order of morphological and molecular cladograms is not as important as is congruence between inferred macroevolutionary transformations at the taxon level as caulistic elements on an evolutionary tree. Unsampled paraphyletic branches can affect perceived progenitor-descendant relationships and may be inserted in a molecular tree to help explain lack of congruent caulistic inferences without affecting calculated branch order. Integrable and non-integrable analyses must be combined for scientific completeness. Support for inferred macroevolutionary transformations may be estimated from either the amount of present-day paraphyly in densely sampled, related groups or from clade support and nearest neighbor interchange.

Key words: Evolutionary systematics, integrable analyses, macroevolutionary transformation, paraphyly.

There has been a misunderstanding in the present conflict between proponents of phylogenetic and classical evolutionary systematics regarding paraphyly. It has been stated (Santos & Faria, 2011: 67) that there is a war between advocates of strictly molecular or strictly morphological systematics. Evolutionary systematics, however, promotes pluralism in analytic methods and cherishes molecular cladograms as informative of aspects of evolution. It is how phylogeneticists interpret exemplars in molecular cladograms as representative of taxonomic units that is the basis for the dispute.

The molecular cladogram can fairly accurately give a retrodiction of the gene history of each specimen used as an exemplar for a taxon. Gene history is used here as restricted to genetic continuity and isolation events in the ancestral line ending with each *sample*. Branch order of the *taxa* supposedly represented by the specimen exemplars are, however, not directly modeled. Hörandl (2010) has pointed out that molecular data are efficient for reconstruction of descent, but commonly used markers have limited value for recognizing evolutionary groups, while morphological traits that contribute to structure and function are actually involved in selection, adaptation, and co-evolution, and, thus, may be the proper bases for evolutionary grouping in classification.

Zander (2008, 2010a) introduced the concept of taxon mapping where heterophyly (paraphyly or phylogenetic polyphyly of undeniably the same taxon) implies a deep ancestral taxon generating two extant lineages of the same taxon, and this progenitor also generated one or more lineages of different, apophyletic (descendant) taxa (at the same or higher rank) in between the paraphyletic branches. Theoretical macroevolutionary transformations are then synthetic, emergent properties. Looking for deep ancestors linking molecular and morphological inferences is equivalent to the search for "hidden variables" in physics, such as the so-far unsuccessful search for a non-obvious classical explanation for the nonsensical rules of quantum mechanics. Paraphyly may be simple, with one included lineage of a different taxon (Fig. 1a), or extended, with two or more included lineages (Fig. 1b) bracketed by a heterophyletic pair of lineages of the single taxon that establishes the deep ancestor of the same name.

This paper is written in the context of a framework for a post-phylogenetic systematics (Zander, 2013), which attempts to meld alpha taxonomy, morphological cladistics, and molecular systematics under a single scientific theory that explains their incongruencies. Postulation of theoretical caulistic macroevolutionary transformations, as taxonomically named internal nodes, forms the basis for that overarching

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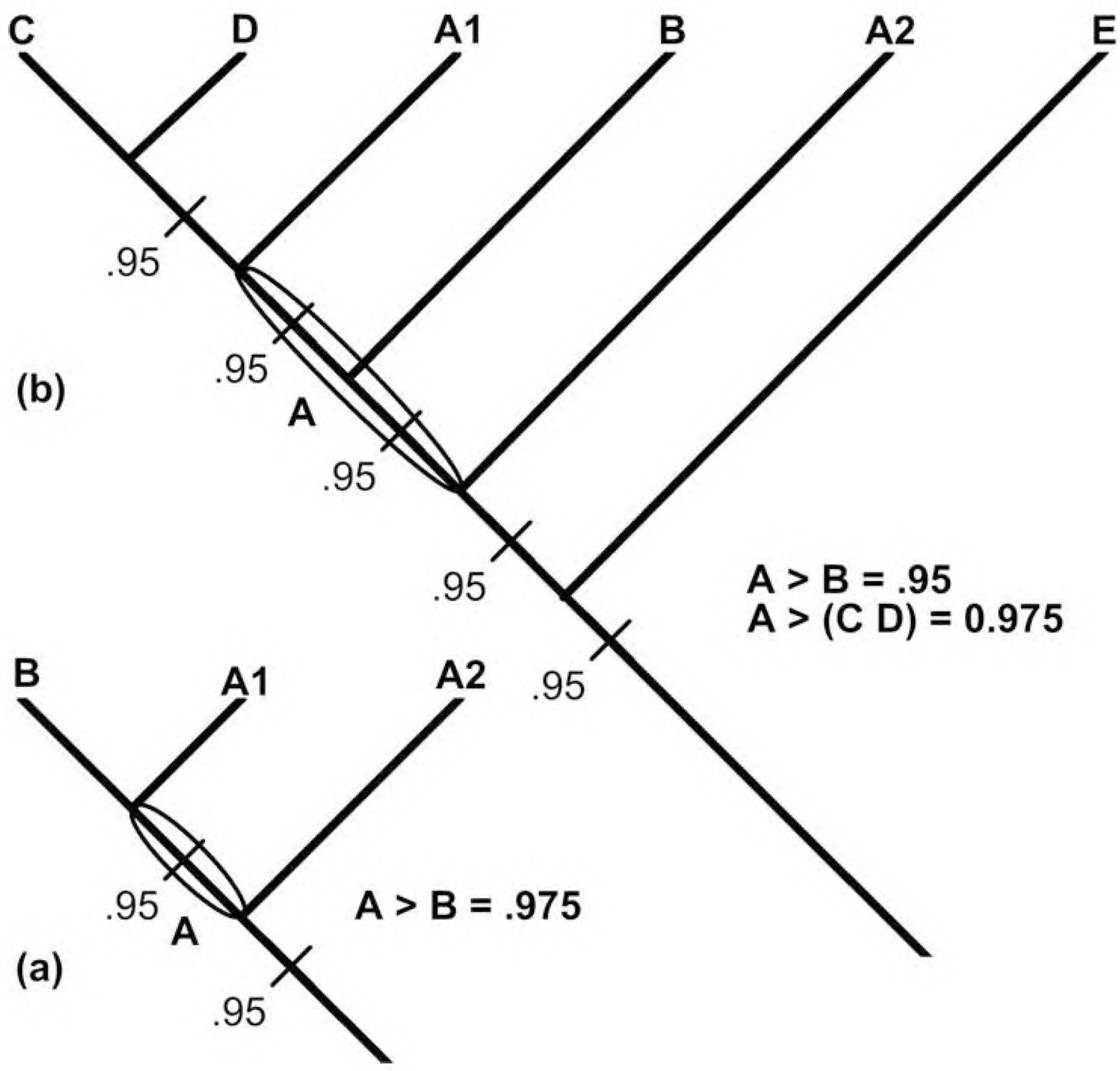


Figure 1. —(a). Simple paraphyly. Support for macroevolutionary transformation A > B through nearest neighbor interchange analysis has one half the uncertainty of support for sister-groups (B, A1). —(b). Extended paraphyly. Both B and (C, D) are "bracketed" by A1 and A2, and, thus, are both descended from implied deep taxon A. Support for transformation A > B is the product of one minus the uncertainty contributed by two calculations, the nearest neighbor interchange of A1 and B, and of B and A2 (see discussion in text). The support for A > (C, D) is calculated as per A > B in Figure 1a.

theory, as macroevolution in phylogenetics is presently a hidden variable. The method has six elements: (1) alpha taxonomy is a set of genetic-algorithm–based heuristics developed over 250 years; (2) cladistic analysis of morphology aids in developing a natural key to taxa based on transformations of weighted conservative characters; (3) molecular systematics establishes genetic continuity and order of isolation events, but not necessarily speciation events, through deep ancestors implied by heterophyly of exemplars; the name and rank of the ancestral taxon is that inclusive of all the heterophyletic exemplars; (4) taxa low in the morphological tree but high in the molecular tree are theoretically ancestral taxa of all lineages in between, while morphological analyses may be reassessed with molecular taxon mapping; (5) "superoptimization" by maximizing theoretical ancestor-descendant hypotheses minimizes superfluous postulated shared ancestors, while biosystematic and biogeographic study through Dollo evaluation at the taxon level provides biological evidence for macroevolutionary transformations; and (6) classification by diagnosable macroevolutionary constraints requires the generalist Linnaean classification system capable of representing to some degree all aspects of evolutionary analysis through taxon-inclusive lists (distinctions) and ranks (similarities). In this paper, the level of support for macroevolutionary transformations at the taxon level is measured, based in part from support for clades.

Distinction between What an Exemplar and a Taxon Represent

An exemplar of a taxon in a molecular cladogram is often the sole representative assigned to that taxon. There is in effect a single sample of the distribution of the potentially variable sequence data for that taxon. This is opposed to the practice of examination of many specimens in classical taxonomy, which allows recognition of the distribution and correlation of conservative and of more or less variable traits. The classical taxonomic method, as Zander (2010a) detailed following Gigerenzer (2007) and Hutchinson and Gigerenzer (2005), uses hard-won informal genetic algorithms resulting in well-tested heuristics called "expertise." Such is not usually associated with explicit rules-based analysis, but the development (Zander, 2008, 2010b) of a logical, effective, overarching theory of macroevolution melding "subjective" (Scotland et al., 2003; Hey, 2009; Yoon,

2009) Linnaean taxonomy with the results of molecular systematics (see Zander, 2010a, for examples) demonstrates the utility of Linnaean classification as the most successful genetic algorithm of alpha taxonomy.

Ideally, the distribution of traits among sampled specimens of the molecular data should match that of the morphological data. Although morphological data have been accumulated over the past 250 years of classical study and distributional variation is well documented in descriptions of at least the well-studied species, this is not true for molecular distributions, which focus on sampling sequences, not specimens. Such sampled sequences may even be from different specimens. Thus, different gene histories may be due to differential lineage sorting or to different, paraphyletic specimens as source of two different sequences. Molecular exemplars rely on classical descriptions for a model of what they represent. Molecular analyses, when they match morphological results, can only be said to be "not incompatible" and never cited as supportive because exemplars are usually insufficient to stand by themselves as well-documented representatives of coherent entities in nature; or, if they do not match, they can only be incongruent and never truly falsificatory. This is because incongruence between taxon-based morphological and specimen-based molecular analyses can be explained with an overarching theory, namely as due to macroevolution—that is, one taxon generating another taxon of the same or higher rank. Rejecting macroevolution is characteristic of structuralism in systematics (Zander, 2010b). Pluralism in method, involving theory and total evidence, is promoted by evolutionary systematics.

Uncertainty Associated with Exemplar Branch Order

Systematics has restricted analysis to integrable (fully solvable) problems (Ekeland, 2006: 80), in particular Markov chains and parsimony analysis, which ideally can mathematically fully predict the past and future, much like Laplace's demon, as a generalized solution. The molecular cladogram is commonly analyzed on a Markovian model in that present-day data are taken as sufficient to retrodict the relevant past (if a large enough data set), and analysis is by a reverse Laplace's demon through coalescent theory. In integrable analyses, such as prediction of the future position of an ideal billiard ball on an ideal elliptical pool table, uncertainty is not increased beyond the initial uncertainty. This in systematics is not totally true in practice as Hudson and Coyne (2002) and Rannala and Yang (2003) have discussed conflicting gene trees generated during the coalescent process by stochastic fluctuation. A molecular tree is precise but reflects only the limited data involved in its construction. The incessant drive for precision makes precision the enemy of accuracy. The genetic continuity and isolation events of a given exemplar specimen's gene history are indeed inferable, but speciation events and macroevolutionary series (named taxa giving rise to named taxa of the same or higher rank) are not directly investigated. The tree of life in molecular phylogenetic analysis remains only a nested parentheses of sampled specimens and has no better resolution than the expected level of paraphyly and extended paraphyly due to unsampled and extinct lineages or molecular strains that would render monophyletic groups paraphyletic if they were known.

To infer macroevolution as named nodes on a tree, less precise data and discursive, inductive reasoning must be involved. The problem becomes nonintegrable and can lead to chaos, much as the position of even an ideal caroming billiard ball is not predictable after about seven bounces off a cushion on a rectangular pool table (Manzotti, 2011) as it transits into Brownian motion-like chaos. The problem is even less certain as data degrade with time since the evolutionary event. A solution to nonintegrable problems (Ekeland, 2006: 97, 103) necessarily involves analysis of individual elements (taxa) with all data available, while periodicity is revelatory of relationships between the elements. The periodicity as applied to systematics, in part, is reflected in shared traits in the context of macroevolution, i.e., Darwin's original concept of descent with modification of taxa. Dayrat (2005) demonstrated that Darwin's tree of life was based on taxon-based progenitor-descendant transformations, not sister-group relationships. In addition, uncertainty is contributed by the use of induction in generating scientific theories of caulistic macroevolutionary transformation, and induction also increases the chance of false conclusions from true premises (Sober, 1991: 20). Increased uncertainty associated with true "total evidence" analysis must be accepted for a complete scientific theory.

Zander (2007) advanced the case for a penalty of 1% in credibility support for all branches of a molecular cladogram to allow for unaccounted assumptions. Zander pointed out that the final product of a Bayesian analysis is properly not the posterior probability, which reflects only the data set, but the Bayes' solution (Kendall & Buckland, 1971), which minimizes risk by taking into account

uncertainty contributed by assumptions not dealt with in the method, model, or data. Sources of uncertainty, none of which are unfamiliar to phylogeneticists, include alignment, wrong gap costs, differential lineage sorting, hybridization, polyploidy, recombination, non-clocklike behavior, rates other than gamma distributed, differences between the results of "total evidence" and evaluations based on separate gene studies, possible strong selection pressure on noncoding promoter sequences, persistent pseudogenes, too few exemplars, endogenous retroviruses, gene conversion, self-correction of flawed DNA, paralogy, codon bias, chloroplast capture and other horizontal gene flow, novel clades, saturation, third codon bias, wrong identifications, long-branch attraction, model insufficiency, and other problems affecting the Bayes' solution. The argument for a 1% penalty on the credible support (i.e., multiply the support by a correction factor of 99%) involves the following: if only 10 of these assumptions affected one branch support one out of 1000 times, then the joint contributed uncertainty is 1%, or 20% of the 5% window of reliability. This seems onerous, yet it can be dealt with by empiric Bayesian analysis of multiple sequence studies (Zander, 2007).

Following the argument that an integrable analysis does not increase initial uncertainty, the initial uncertainty of 1% is simply reflected at all solutions (nodes). This penalty is for error that increases linearly with additional contiguous branch splits. That is, the joint probability of more than one branch is the product of their individual credibility support values. In the case of phylogenetic trees with all branches supported at 99% credibility, only five contiguous internodes (chained clades) anywhere in the tree are acceptable as having branch order being completely correct at a joint probability of 95% (that is, 99% multiplied by itself five times). One percent is a general penalty applied to all support measures for all lineages because the errors affect all approximately equally. The 1% uncertainty is compounded because each node is a separate solution to the integrable analysis.

There is a notion, particularly among mathematicians, that nature follows mathematics (Kline, 1985; Ekeland, 2006). On the other hand, the perceived real features of nature are fuzzy, somewhat indeterminate, and probabilistic due to the influence of complexity and chaos, plus the fact that no phenomenon is fully described by available data. Mathematics is then a kind of approximation, in this case hyper-precise for phenomena that are difficult to encompass with a precise answer, and mathematical solutions thus may be inaccurate for all but the simplest, most well-understood phenomena. The map is not the territory. An exacting bill for services may be wrong. An integrable solution may produce a simple and repeatable classification, but the implied evolutionary relationships may be scrambled.

Inferred Macroevolutionary Transformations

Rieseberg and Brouillet (1994) estimated at least 50% of all plant species are generated through local geographic speciation and are, therefore, paraphyletic. Funk and Omland (2003) found clear species-level paraphyly or polyphyly in 23% of more than 2000 species sampled. According to Aldous et al. (2011: 322) about 63% of extant species have extant ancestors. Frey (1993) decided that paraphyletic scenarios, including local geographic speciation, are common or even the rule, while Gurushidze et al. (2010) supported the idea that pseudoextinction (disappearance of a progenitor taxon after generation of two daughter taxa) is rare. Thus, there are doubtless many cryptically paraphyletic taxa that have one branch unsampled, for instance because of lack of time, funding, or computer limitations, or the lineage is extinct or represented only by difficult-to-obtain or very old specimens. The sampled lineage only appears to be monophyletic. The percentage of paraphyletic taxa at any one time in the past, the past measured by each split in a cladogram, may be judged by the percentage of paraphyletic taxa in the present. If 10% of the taxa represented by exemplars in a cladogram are paraphyletic, then the chance of any presently monophyletic internode in the cladogram representing a paraphyletic taxon is 10%, one branch of the paraphyly assumed probably unsampled. That percentage, however, is not the same as the percentage of all taxa being initially paraphyletic, which may be nearly all of them, or the percentage of taxa with long-lasting macroevolutionarily generative paraphyletic lineages that are unsampled today. In addition, given little or no information on the percentage of true pseudoextinction events, an attempt to infer a progenitor at each and every node in a cladogram seems justified. This is "superoptimization" because it decreases the number of unnamed shared ancestors that must be postulated in addition to optimization of traits on the tree. This may seem counterintuitive to "tree-thinking" phylogeneticists for whom shared ancestors are never named (beyond, perhaps, as that taxon including all exemplars but the outgroup). As Einstein said to Heisenberg (Gilder, 2008: 87): "It is theory which first determines what can be observed." Phylogenetic theory is blind to caulistic macroevolutionary transformations at the taxon level.

At times it is necessary to postulate unsampled paraphyly in a molecular tree to explain rather certain relationships among morphological taxa. The attainment of "reciprocal monophyly" may be the perfection of cladistic relationships but is also the loss of macroevolutionarily important information. Morphological taxa are often well supported. The amount of data is commonly comparable between morphological and molecular studies, or even much larger in morphological studies, contrary to assumptions in the literature. Consider this contrived scenario: (1) A morphological cladogram is based on descriptions of 50 taxa, each taxon consisting of 500 specimens studied over many years by modern generations of taxonomists, based on 20 conservative traits (and 20 or more other, less conservative traits). Thus 20 times 500 times 50, or 500,000 data points are involved referring to *taxa*. A weighted analysis emphasizing known conservative traits yields the cladogram of taxa (((A,B)C)D,E). Superoptimization, let us say, supports the morphological analysis by indicating that taxon A is probably the ancestor of both B and possibly also C. (2) A molecular analysis of specimens representing the same taxa is done, based on, say, 50 specimens and 1000 phylogenetically informative traits. Thus, 50,000 data points are involved for the 50 *specimens*. A parsimony or Bayesian analysis yields the cladogram of specimens (((A,C)B)D,E). The two cladograms do not actually conflict because their theoretical macroevolutionary transformations are the same. The two analyses infer two different cladistic facets of evolution: one, the overall synchronic expressed trait relationships of taxa and the other, the synchronic relationships of specimens based on inferred genetic continuity and isolation events. It is not appropriate to simply ignore the morphological cladogram or to map traits atomistically on the molecular cladogram. Scientifically, no violence is done to logic and no information is lost if one postulates an unsampled paraphyletic branch of A occurring below B on the molecular cladogram, supporting the inference from superoptimization of the morphological cladogram that A is the ancestor of B, and also C. Refusing to engage in scientific theorization about macroevolution is a major problem with phylogenetic analysis, yet such theorization yields testable hypotheses of major import.

Support Measures for Macroevolutionary Transformations at Taxon Level

The present-day branch order of exemplar specimens is not in question because phylogenetic branch order is valuable to the extent it helps infer macroevolutionary transformation of taxa. Unsampled (e.g., extinct) paraphyly can affect the probability of which ancestor is, for instance, the most basal in a caulogram. Investigating the possibility of identification of an ancestral taxon as a node on a cladogram involves evaluating the amount (percent of taxa with exemplars exhibiting paraphyly) and extent of scatter (distance between heterophyletic exemplars) of paraphyly in the cladogram as a whole, and also data not in the phylogenetic data set, including discursive reasoning about direction of evolution involving Dollo's law (Gould, 1970) and biogeographic considerations (Zander, 2008). Although many specimens have been sampled among taxa of higher rank, no direct, formal evaluation (e.g., Student's *t*) of skewness or multimodalities among the molecular data has been done.

Given that good sampling of taxa (e.g., many specimens from many localities and habitats that sample a possible multiplicity of intrataxon populations) is rare, we must use rules of thumb for the prevalence of paraphyly. In general, at least simple paraphyly is here considered extremely common in the past and mostly is unsampled. Extended paraphyly (or phylogenetic polyphyly) is less common but seems not uncommon judging from published molecular trees in the literature.

In the case of *extant* paraphyly, support measures for inferred macroevolutionary events at the taxon level can be derived from support measures for exemplar specimen branch order on a molecular tree. (Note that paraphyly on a morphological tree is also possible and informative, although not much investigated nowadays.) In the contrived rooted cladograms (Fig. 1a, b), all clades are supported at 0.95 posterior probability, a common measure of good support. In simple paraphyly, as in Figure 1a, support for specimen B (representing taxon B) being derived from caulistic deep taxon A, implied by paraphyletic specimens A1 and A2, can be inferred from nearest neighbor interchanges (NNI). NNI between (B, A1) and A2 would not affect A > B (i.e., B derived from ancestor A) if A1 interchanged with A2, but would if B interchanged with A2. Because the support for (B, A1) is 0.95, we can estimate half the uncertainty, or a support value of 0.975 for A > B. This is a simplification done in lieu of evaluating all the most probable branching patterns. The chance of B interchanging lower in the cladogram (assuming more clades toward the root) is small, at most one half of 0.05 times 0.05, or 0.0013, which, though finite, does not much lower the support for A > B.

In another scenario, that of extended paraphyly (Fig. 1a), both B and (C, D) are bracketed by A1 and A2, these last being two exemplar specimens of inferred ancestral taxon A. There are then two

lineages that apparently derive (descend from) the implied taxon A. The support for the macroevolutionary transformation A > B depends on NNI governed by support measures between A1 and B, and A2 and B. NNI between B and A2 eliminated support for A > B, but switching A2 with the other lineage ((C, D) A1) does not. Thus, 0.975 is support from this NNI analysis. Switching of A1 with B also eliminates support for A > B, but switching (C, D) with B does not, thus 0.975 is the other half of the analysis. Both must be true at the same time for A > B, so the joint probability of A > B is 0.975 times 0.975, or 0.95.

The second apparent macroevolutionary transformation (in Fig. 1b) is then A > (C, D). The NNI analysis is that if (C, D) switches with B, there is support, but if A1 switches with B, there is no support, thus support for A > (C, D) is 0.975 (half the 0.05 uncertainty of the support value). The name of the implied caulistic taxon for the immediate shared ancestor of C and D would be the lowest ranking taxon that includes both C and D.

A deep ancestral taxon may be inferred by cross-tree heterophyly of a distal exemplar on a molecular cladogram and that same taxon more basal in a morphological cladogram of the same taxonomic group. This is done by postulating an unsampled (e.g., extinct) lineage inserted into the molecular cladogram in the same more basal position of the morphological cladogram (Element 4 of the framework above). An example is the position of the moss genus *Erythrophyllopsis*, very low in a morphological cladogram (Zander, 1993: 47) but rather high in a molecular cladogram (Werner et al., 2004) as a kind of bryological "coelacanth." The level of clade support for the position of an inserted theoretical lineage must be high (say, 95% posterior probability) if the morphological cladogram otherwise roughly approximates the molecular cladogram and there are similar taxa nearby (implying a similar generalist ancestor, as in the case with *Erythrophyllopsis*) in the morphological cladogram. In addition, if there are no alternatives, Cohen's (1994) arguments against unremitting calculation of superfluous support values then applies. Calculation of support for evolutionary transformations from an inferred deep ancestor can be based on the two heterophyletic branches. One must remember that the integrable molecular cladogram is an incomplete theory because it details (as a discovery process) only inferred genetic continuity and isolation events, not descent with modification of taxa. The addition of inductive nonintegrable inferences may limit certainty but is scientifically sound and theoretically complete.

One can point out here that the level of support for the existence of a deep ancestral taxon gauged from extant paraphyly also helps judge the level of existence of possible unsampled deep ancestral taxa that render non-paraphyletic lineages ambiguous as to branch order. Simple paraphyly implies a deep ancestral taxon that does not change cladistic branch order, assuming no name changes associated with the principle of holophyly, but extended paraphyly will change taxon branch order. As a rule of thumb from estimates of Aldous et al. (2011), unsampled simple paraphyly may be assumed to be about 50%, and from observed published cladograms of taxically fairly well-sampled studies, unsampled extended paraphyly may be about 10% of that, or 5%. A macroevolutionary transformation associated with extended paraphyly changes branch order if an unsampled lineage is two or more nodes below the extant lineage of the same taxon. Any monophyletic lineage then has a 5% chance that it is subtended by such an order-changing unsampled lineage. In the absence of any other information, each Bayesian support measure for a monophyletic branch must be reduced by 95%.

Discussion

Evolutionary systematics promotes the use of all data and all analyses, both integrable and non-integrable. In that Markov chain analysis is np-hard, Hastings–Metropolis sampling speeds the computation. Likewise, parsimony analysis has a heuristic sampling option for "large" (more than 20 OTUs) data sets. Although such analyses are valuable sources of particular evolutionary information, there is no direct inference of macroevolution, that is, derivation of one named taxon from another named ancestor at the same taxonomic level, or higher (e.g., species to species or species to genus). It is when the specimens that are analyzed molecularly are treated as exemplars (representatives) of taxa that a confusion of integrable and nonintegrable analyses begins. The taxa are determined by nonintegrable classical circumscriptions involving informal heuristics, i.e., scientifically informed intuition refined over 250 years of alpha taxonomy. The data used do fade with time of establishment, yet many data on different traits allow a kind of triangulation that well distinguishes taxa on the basis of one or another of several valid species concepts.

Following Ekeland's (2006) example of the rectangular pool table that yields nonintegrable predictions of a pool ball's path, there are two paths that are in fact integrable, namely bouncing back and forth orthogonal to the sides. This periodicity is a

source of long-term predictivity. An analogue in classical systematics is the conservative trait, which is apparently resistant to adaptive pressures or which mutates rarely. Thus, in combining integrable and nonintegrable analyses, we rely on the informational content of conservative traits to fade the least as time passes. When specimens used in molecular analysis are treated as taxa, the conservative traits are impressed on the analysis. But phylogeneticists should not rely on conservative traits to reflect phylogenetic monophyly of taxa and at the same time reject such traits in the case of paraphyly. Likewise, one should not postulate homoplasy or independent derivation with no further explanation when a theory (macroevolution) is available that explains such as joint derivation from an inferred deep ancestral taxon.

When studies of molecular and morphological branch orders are quite dissimilar, the classical taxa are re-evaluated to see if they really belong in this study or group, or if they are too heterogeneous or homogeneous, or if a morphological cladogram is correctly weighted to reflect the relative importance of conservative traits. If the conclusion is that there is no reason to change morphologically based taxa, the molecular cladogram is then evaluated to see if insertion of a theoretical unsampled paraphyletic lineage may account for a deep ancestor that will explain the differences in branch order. The number of inferred unsampled paraphyletic lineages should not exceed 63% of the number of taxa represented by the exemplars plus all the unnamed shared ancestral nodes (see Aldous et al., 2011: 322), without a clear reason. Inferred empirically unsampled paraphyletic lineages should not be inserted farther from the extant lineage than is reasonable given the scatter of extended paraphyly in well-sampled (taxon-wise) molecular cladograms of at least related taxa. The goal is not necessarily forcing morphological and molecular cladograms to be congruent but to develop a theory of shared caulistic ancestral structure that explains both best. This is much like Haack's (1993: 81) analogy of the crossword puzzle that is solved by mutual support from two rather different belief or experiential systems.

Both morphological and molecular cladograms may be modified to meld with each other in sharing the same macroevolutionary transformation because each is vulnerable. Morphological data are difficult to weight without a thorough analysis; such weights may not be generalizable to a full cladogram; and trait parallelism or re-occurrence of ancestral traits is well known (Zander, 2007) though amenable to Dollo evaluation at the taxon level. Molecular analysis is of specimens, not taxa, and unsampled heterophyletic lineages are doubtless common given the usual prevalence of paraphyly and extended paraphyly in well-studied densely sampled groups. Hörandl (2010) discussed incomplete sampling of specimens and sequences in the context of deep time markers and implied that morphological and molecular analyses must be expected to remain incongruent in some respects. These vulnerabilities, in fact, help triangulate shared caulistic transformations through application of macroevolutionary theory. Support measures for inferred deep ancestral taxa may be calculated from the estimated extent of present-day paraphyly in densely sampled groups, and NNI analysis of clade branch order of present-day and theoretical paraphyly.

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PANDAS, PLANTS, AND PEOPLE[1,2]

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Abstract

Plants are essential for the survival and sustainability of both humans and wildlife species around the world. However, human activities have directly and indirectly affected almost all plants, which in turn have produced cascading effects on humans and wildlife through disruption of crucial ecosystem services and wildlife habitat. Understanding such complex interactions is crucial for developing better policies that reconcile the needs of an ever-growing human population with biodiversity conservation. Using the coupled human and natural systems (CHANS) framework, this article synthesizes research on the complex interactions of plant species, giant pandas, and people. The CHANS framework is particularly useful for uncovering key patterns and processes behind plant-animal interactions modified by human activities. Our synthesis shows that many human factors, including socioeconomic and demographic, together with other factors (e.g., projected global climate change), exhibit reciprocal interactions with pandas and the plant species that comprise their habitat. Although substantial efforts have been made to preserve plants and wildlife, much work still remains to be done, including the expansion and more effective management of protected areas, use of native plant species in reforestation/afforestation programs, and active participation of local residents in conservation actions.

Key words: Bamboo, China, coupled human and natural systems (CHANS), ecosystem services, endangered species, giant pandas, protected areas, wildlife.

Plants provide essential sources of food, fiber, medicine, and other important goods and services for humans (Díaz et al., 2006). They also constitute crucial components of wildlife habitats (Tuanmu et al., 2011), offering not only food to herbivorous wildlife species but also shelter to herbivorous and carnivorous wildlife species across many different ecosystems (Taylor et al., 2004; Nilsson & Wardle, 2005; Gilliam, 2007). However, human activities have converted much of the natural landscapes to human-dominated landscapes and have led to biodiversity loss worldwide (Vitousek et al., 1997; Sala et al., 2000; Pereira et al., 2004; Waltert et al., 2004; Lepczyk et al., 2008). This loss is usually expressed as species extinction at local, regional, and global scales and homogenization of regional and continental biotas. The rate of species extinction due to human activities is between 1000 and 10,000 times faster than background rates, i.e., those without human causes (Hilton-Taylor, 2000; Pimm & Raven, 2000; Ceballos et al., 2010). As a result, according to criteria of the International Union for Conservation of Nature (IUCN, 2001), estimates of the percentages of plant species worldwide that may be threatened with extinction range from ca. 22% to as many as 50% (Pitman & Jørgensen, 2002; Baillie et al., 2004). The loss of plant biodiversity poses enormous direct and indirect consequences for humans as well as wildlife species and, ultimately, the structure and function of ecosystems (Hooper et al., 2005). Therefore, the loss of plant biodiversity is of great concern not only for ethical and aesthetic reasons but also for its cascading effects on entire ecosystems and the goods and services they provide to humanity.

The loss of biodiversity has inspired many conservation actions, such as the establishment of protected areas and implementation of conservation policies. Protected areas are supposed to be areas where human activities are limited or controlled (DeFries et al., 2007). By 2011, there were over 130,700 protected areas, covering more than 24 million km^2 of surface area on the earth (IUCN, UNEP-WCMC, 2012). Although their creation constitutes a cornerstone of biodiversity conservation (Margules & Pressey, 2000), biodiversity inside these areas is not necessarily better protected than outside them (Liu et al., 2001; Caro, 2002; Parks & Harcourt, 2002; Meir et al., 2004; Viña et al., 2007), because

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many people inside and outside the protected areas still use them in a variety of ways, such as for harvesting timber, agriculture, mining, and tourism (Liu et al., 1999b). They are also exposed to the potentially negative effects of human-induced climate change (IPCC, 2007).

The world's biodiversity conservation challenge is well represented by China. Due to its enormous variation in climatic, topographic, and geologic features, China is one of the world's 17 mega-diverse countries (Mittermeier et al., 1997) and ranks third in the world (after Brazil and Colombia) in the number of known plant species (Liu et al., 2003b). China's flora is extremely rich and diversified, with around 33,000 vascular plant species in more than 3000 different genera and ca. 334 families of angiosperms, ca. 42 genera and 11 families of gymnosperms, 231 genera and 63 families of pteridophytes, and ca. 500 genera and 106 families of bryophytes (Lu, 2004; Wang, 2004; Wu et al., 2004a; Wu et al., 2004b). More than 10,000 higher plant species are endemic to China (Fu, 1992; Pitman & Jørgensen, 2002), with ca. 243 genera found only in China (Ying & Zhang, 1994). In addition, although not endemic, many plant genera have been recorded predominantly in China, including the 650 species of the genus *Rhododendron* L. (of the 1025 distributed worldwide) (Wu et al., 2003, 2004b). This high diversity is at high risk mainly due to deforestation (e.g., for timber and fuel wood), as well as the conversion of both forests and grasslands into croplands and urban areas. While China has been occupied by humans for millennia, the tremendous loss and fragmentation of natural ecosystems have been particularly drastic from the 1950s onward (Dinerstein & Wikramanayake, 1993; Liu, 2010). The huge destruction of China's ecosystems began to receive attention by government authorities due, in part, to the occurrence of natural disasters and the decrease in crop productivity associated with soil erosion and desertification. Therefore, starting in the 1970s, the government began to implement a series of afforestation and reforestation programs that have resulted in a progressive increase of forest cover over the last 40 years (Liu et al., 2013b). Yet nearly all of these new tree plantations, which are replacing previously natural forests, have been mono-specific and often consist of fast-growing exotic species (Viña et al., 2013) that greatly diminish the biodiversity value of the original forests (Xu & Wilkes, 2004). In addition, the government has established many protected areas at an impressive rate (Liu & Raven, 2010). By the end of 2011, a total of 2640 nature reserves were established covering ca. 14.9% of the land surface of China (Ministry of Environmental Protection, People's Republic of China, 2011). Many endangered and charismatic species, such as the giant panda (*Ailuropoda melanoleuca* [David, 1869]), the South China tiger (*Panthera tigris* subsp. *amoyensis* [Hilzheimer, 1905]), and the Asian elephant (*Elaphas maximus* [Linnaeus, 1758]), have been used as surrogates for the establishment of these nature reserves. The giant panda is particularly interesting because it is considered an icon of biodiversity conservation not only in China but in the whole world (Mackinnon & De Wulf, 1994; Liu et al., 2001; Loucks et al., 2001).

Almost all ecosystems, including protected areas, constitute coupled human and natural systems (CHANS) (Liu et al., 2007a, 2007b) in which humans and natural components interact with each other directly or indirectly. Thus, understanding and conserving plants and wildlife require a new approach that treats humans, plants, and wildlife as parts of an integrated system. To illustrate the CHANS approach (Liu et al., 2007a, 2007b), in this article we focus on complex relationships among giant pandas, plants (the tree and bamboo species pandas depend on), and humans. We first outline a conceptual framework of CHANS and then highlight the major components and their interrelationships, using results mainly from our research group and collaborators.

Conceptual Framework

The CHANS approach brings together theoretical foundations (e.g., social norms, social networks, ecological succession) and analytical techniques (e.g., remote sensing, geographic information systems, systems modeling) from diverse disciplines, including those from ecological and social sciences, to understand complex systems (Liu, 2001; Liu et al., 2007a; McConnell et al., 2011). The approach is, therefore, well suited for understanding pandas, the plants they depend on, and humans, which affect both pandas and plants. In this article, we conceptualize the integrated system as consisting of four main components: giant panda habitat, plants, local residents, and policies, all of which, in turn, are influenced by macroscale factors, including climate, natural disasters (e.g., earthquakes, landslides), and telecoupling processes that operate over distances (Liu et al., 2013a; Liu, 2014) (Fig. 1).

Each of the components in our framework both influences and is influenced by the other components. For instance, the abundance, distribution, and diversity of plants (i.e., tree and understory bamboo species) are influenced by people (e.g., local

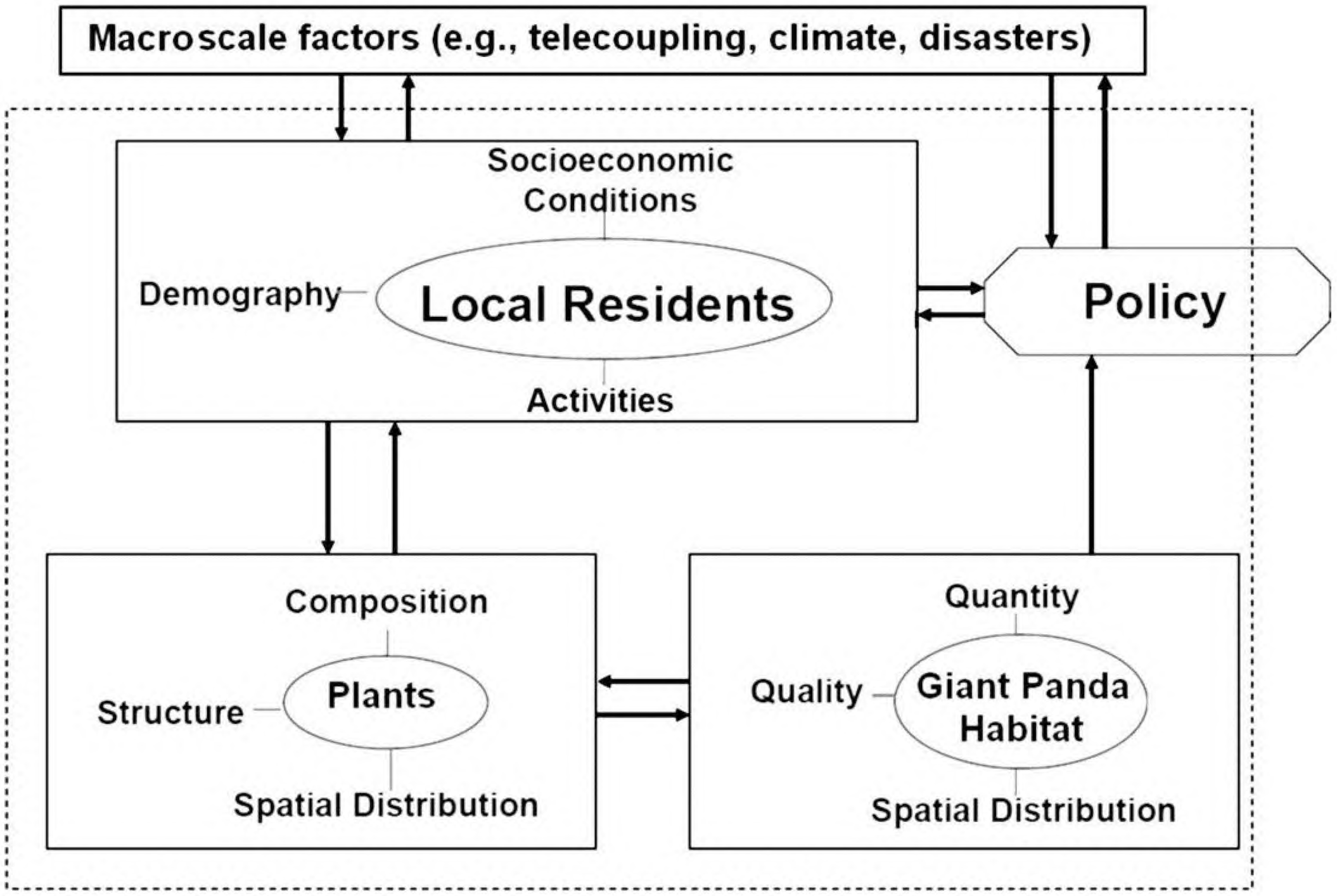


Figure 1. Conceptual framework of coupled human and natural systems (CHANS) for synthesizing information on the relationships among pandas, the tree and bamboo species they depend on, and humans (including conservation policies). Arrows represent the direction of influences among different system components, with those going in both directions representing feedbacks. The dotted line represents the permeable boundary of CHANS.

residents) through different activities such as farming, cutting trees for timber or fuel wood, collection of bamboo shoots, and tree and bamboo plantation (State Forestry Administration, 2006). All these activities modify the habitat suitability of pandas, thus affecting their abundance, distribution, and behavior. While hunting panda individuals is strictly prohibited and the sanctions imposed are drastic enough to discourage this practice, some illegal hunting still occurs, although it is mostly unintended (e.g., pandas may be inadvertently captured by wild boar snares; Lü & Kemf, 2001). Nevertheless, humans have other direct effects on the abundance, distribution, and behavior of pandas, since human settlements pose obstacles to dispersal not only within nature reserves, but also among nature reserves and mountain regions (Xu et al., 2006). Therefore, humans and their activities are at odds with the survival of pandas and of the tree and bamboo species they depend on (Liu et al., 2004).

In turn, the changes in the abundance and distribution of plants and pandas provide feedback to humans through changes in the accessibility to timber and non-timber forest products (e.g., bamboo shoots) and motivate the development and implementation of conservation policies that act both directly (e.g., preventing timber extraction and fuel wood collection; spurring tree planting) and indirectly (e.g., incentives to use alternatives to fuel wood, such as electricity). These policies constitute feedback mechanisms through which changes in the abundance and distribution of plants and pandas may be reduced. In short, policies influence human attitudes and activities, which in turn change panda habitat. Changes in panda habitat may prompt the government to develop and implement new policies. These interrelationships result in numerous feedback loops.

Finally, the relations and feedbacks among the four main components in the framework (Fig. 1) are influenced by macroscale factors. These include changes in climate (IPCC, 2007; Tuanmu et al., 2013), the impact of natural disasters such as earthquakes (Viña et al., 2011; Zhang et al., 2011), and telecoupling processes (Liu & Yang, 2013) such as labor migration and tourism, which in many cases have opposing effects.

Giant Pandas

The historical distribution of the species at one time covered an area of ca. 2.2 million km^2

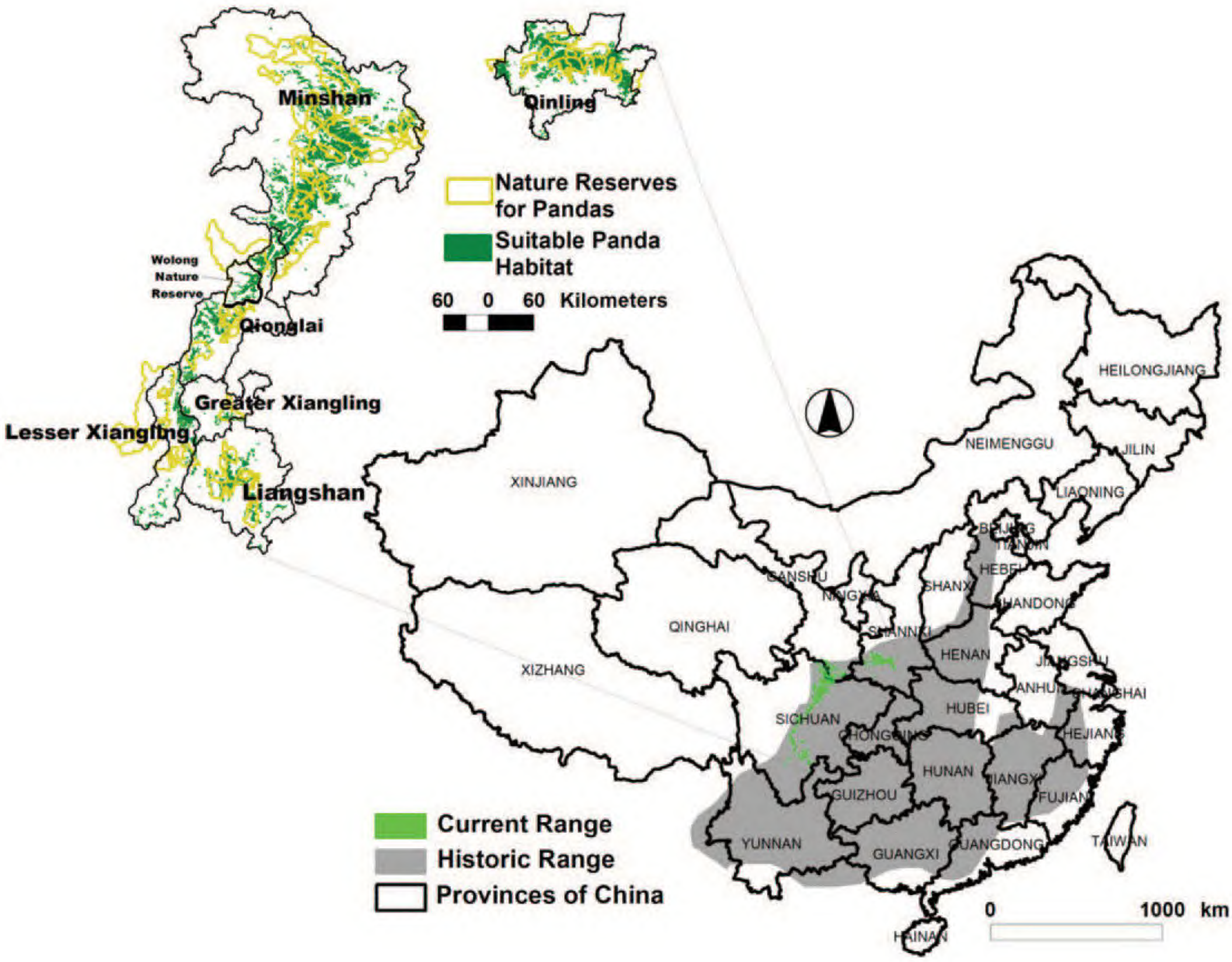


Figure 2. Spatial distribution of the historical (gray) and current (green) geographic ranges of the giant panda (Mackinnon & De Wulf, 1994; Reid & Gong, 1999; Hu & Wei, 2004; State Forestry Administration, 2006; Viña et al., 2010). Also shown are the six mountain regions where pandas currently survive, as well as the 63 nature reserves (including the flagship Wolong Nature Reserve) established for the conservation of the pandas and their habitat.

throughout 19 provinces of China, northern Vietnam, and northern Myanmar (Schaller et al., 1985; Pan et al., 2001) (Fig. 2). However, by the early 1800s this distribution was reduced to only five provinces in southwestern China comprising ca. 262,000 km^2, which was further reduced to ca. 124,000 km^2 by the early 1900s (Zhu & Long, 1983; Reid & Gong, 1999). The current distribution comprises only ca. 21,000 km^2 within six mountain regions (i.e., Qinling, Minshan, Qionglai, Greater Xiangling, Lesser Xiangling, and Liangshan) in three provinces (Gansu, Shaanxi, and Sichuan) of southwestern China (Mackinnon & De Wulf, 1994; Reid & Gong, 1999; Hu & Wei, 2004; State Forestry Administration, 2006; Viña et al., 2010) (Fig. 2).

In terms of the total number of pandas, no estimates before the 1970s (when range-wide censuses started to be performed) are available, although expert opinion suggests that the population could have been ca. 3000 individuals during the 1950s (Hu, 2001). The first census in the 1970s estimated that ca. 2400 individuals survived in the wild, and the second census in the 1980s estimated a decline of more than 50%, to ca. 1100 (Hu, 2001). The third census (performed between 2001 and 2003) estimated the total number of giant panda individuals at around 1600 (State Forestry Administration, 2006). The increase in the number of pandas between the second and the third censuses has been attributed mostly to larger regions surveyed and better techniques used to assess the number of individuals living in a region during the latter census, rather than to an actual increase in the panda population, although such an increase cannot be completely rejected (State Forestry Administration, 2006).

Pandas are extreme dietary specialists; 99% of their diet is composed of understory bamboo species (Schaller et al., 1985). Adaptations to a strict bamboo diet include an enlarged wrist bone to allow for gripping bamboo stems and a large skull and jawbone for mastication of tough plant material (Schaller et al., 1985). However, the panda's short digestive tract

(characteristic of a carnivore) together with a lack of gut microflora suitable for breaking down the cellulose contained in their diet allows the pandas to digest less than 20% of their food intake (Schaller et al., 1985). To respond to these digestive constraints, the pandas have developed behavioral adaptations such as maintaining low energy expenditures (e.g., pandas prefer areas with gentle slopes for ease of movement) and selecting different bamboo species and plant parts with different nutritional characteristics that change along the year (Schaller et al., 1985). Because understory bamboo species in the forests of southwestern China are located at particular elevation ranges, the selection of different bamboo species along the year makes pandas elevation migrants (Linderman et al., 2004). Since the understory bamboo species require forest cover for shade, and pandas also depend on forest cover for shelter (Liu et al., 1999b, 2001), there is a tight relationship between pandas, understory bamboo, and tree species.

Plant Species in the Panda Geographic Range

The current geographic range of the giant panda is characterized by high mountains and deep valleys with elevations ranging from ca. 70 m to more than 6000 m. The drastic elevation gradient combined with complex terrain, geology, and soils is responsible for a high biological diversity, including more than 6000 species of plants in more than 1000 genera (IUCN, 2006). The region also features more than 100 species of mammals in 25 families, and ca. 400 species of birds in 45 families (Reid & Hu, 1991; Taylor & Qin, 1993b; IUCN, 2006). In fact, the region comprises one of the world's top 25 biodiversity hotspots (Myers et al., 2000; Mittermeier et al., 2004).

The vegetation is dominated by evergreen and deciduous broadleaf forests at intermediate and lower elevations and subalpine coniferous forests at higher elevations. Forests occupy around one third of the current panda range, with coniferous, broadleaf deciduous, and mixed coniferous/broadleaf deciduous forests accounting for ca. 48%, 32%, and 20% of the forest cover, respectively (Fig. 3). Tree species diversity in these forests is influenced by elevation and shows conspicuous declines in the number of tree species with an increase in elevation, particularly above 2500 m (Viña et al., 2012).

A total of 115 tree species were found in 104 field plots randomly distributed within an elevation range of 1000–3000 m in broadleaf deciduous, coniferous, and mixed forests across the Qinling Mountains region (Fig. 2) (Viña et al., 2012). Dominant tree species (i.e., occurring in $> 20\%$ of the field plots) in this mountain region were *Quercus aliena* Blume, *Betula albosinensis* Burkill, *Prunus scopulorum* Koehne, *Toxicodendron vernicifluum* (Stokes) F. A. Barkley, and *Pinus armandii* Franch. (Viña et al., 2012). In a similar study in the Wolong Nature Reserve (the first and one of the largest giant panda nature reserves, established in 1975 and encompassing ca. 200,000 ha in Sichuan Province) located in the Qionglai Mountains region (Liu et al., 1999a) (Fig. 2), a total of 81 tree species were found in 62 field plots distributed along four transects ranging in elevation from 1500 to 3200 m (unpubl. data). Dominant tree species (i.e., occurring in $> 20\%$ of the field plots) in this region were *Acer laxiflorum* Pax, *Salix dolia* C. K. Schneid., *Prunus pilosiuscula* (C. K. Schneid.) Koehne, *B. albosinensis*, *Abies faxoniana* Rehder & E. H. Wilson, *Corylus ferox* Wall. var. *tibetica* (Batalin) Franch., and *Sorbaria arborea* C. K. Schneid.

Bamboo species are a particularly conspicuous feature in the understory of the forests of the panda geographic range. Although pandas can feed on more than 30 understory bamboo species found across their geographic range, they prefer only ca. 14 spatially dominant ones (Table 1). In the Qinling Mountains region the understory canopy is dominated by three bamboo species: *Fargesia qinlingensis* T. P. Yi & J. X. Shao, *Bashania fargesii* (E. G. Camus) Keng f. & T. P. Yi [= *Arundinaria fargesii* E. G. Camus], and *F. dracocephala* T. P. Yi (Viña et al., 2012), while in the Wolong Nature Reserve (Qionglai Mountains region), the dominant bamboo species are *F. nitida* (Mitford) Keng f. ex T. P. Yi, *F. robusta* T. P. Yi, and *B. faberi* (Rendle) T. P. Yi [= *A. faberi* Rendle] (Linderman et al., 2005b; Viña et al., 2008; Tuanmu et al., 2010). Because there is usually only one dominant understory bamboo species at a given location (Johnson et al., 1988; Reid et al., 1989; Linderman et al., 2005b, 2006; Viña et al., 2008; Tuanmu et al., 2010; Viña et al., 2012), changes in their abundance and spatial distribution (e.g., through bamboo harvesting; bamboo die-offs as a result of mass flowering occurring at time intervals from 16- to 90-year cycles depending on the species; cf. Table 1) could cause food shortages for the pandas (Schaller, 1987; Reid et al., 1989).

The spatial distribution of understory bamboo species is influenced by many factors, including panda utilization and interactions with other plant species. Regarding the former, Pleistocene fossil records (Zhu & Li, 1980) indicate that the giant panda and the bamboo species composing their diet had plenty of time to develop co-evolutionary traits

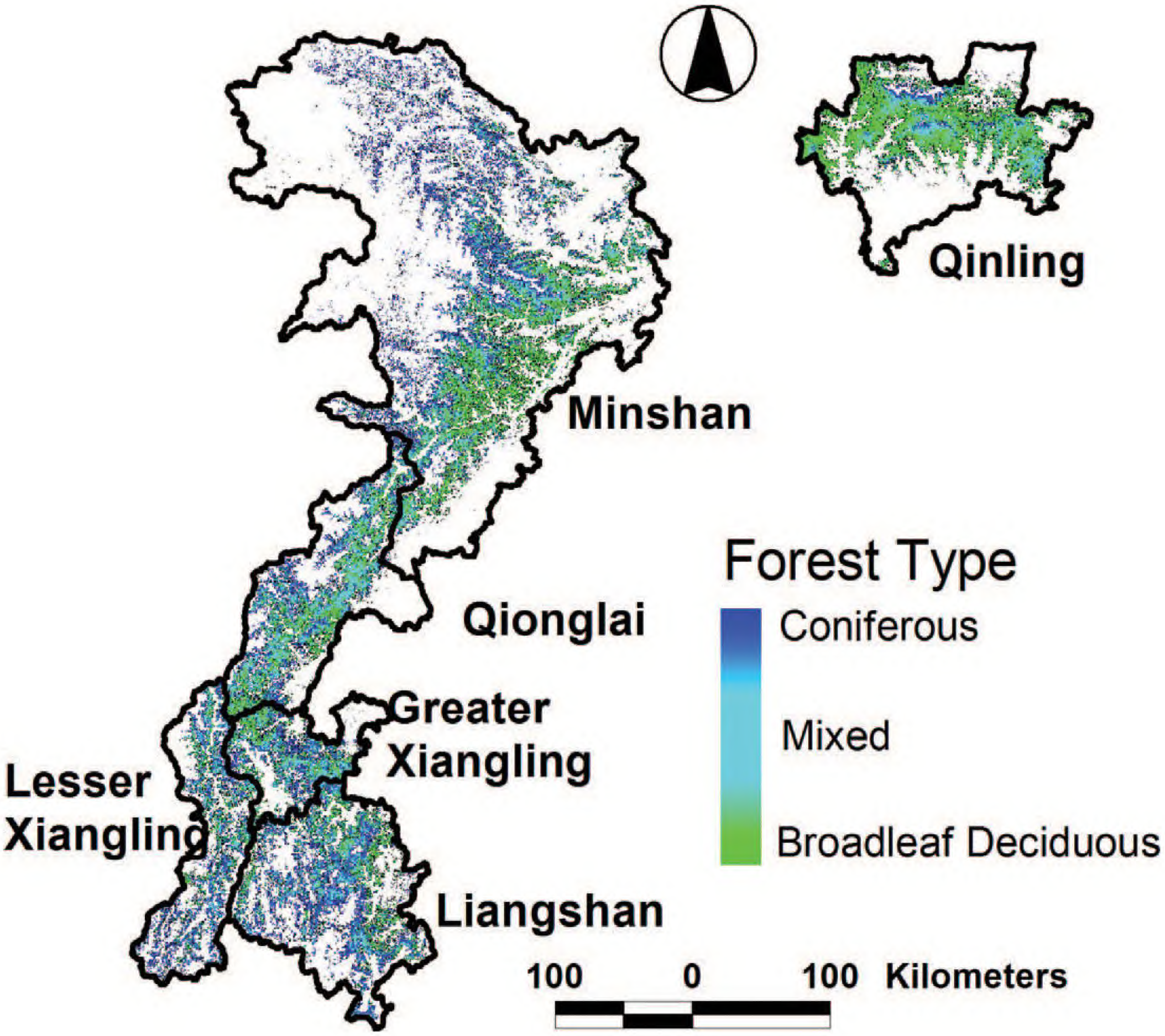


Figure 3. Distribution of coniferous, broadleaf deciduous, and mixed coniferous/deciduous forests in the mountain regions (in 2007) comprising the geographic range of the giant panda. Coniferous forests occur mostly at higher elevations, while mixed coniferous/deciduous forests and broadleaf deciduous forests occur more so at intermediate and lower elevations, respectively. Some coniferous forests also occur at intermediate elevations but are usually planted assemblages.

that ensured their survival, e.g., by reducing potentially negative effects of overgrazing. Nevertheless, under the current drastically reduced areas of suitable habitat, the degree to which bamboo overutilization potentially results in a decline in both quality of bamboo stands and viability of the panda populations needs to be assessed. Studies conducted in Wolong Nature Reserve have found that *Fargesia robusta* had a low yearly recruitment rate, reflecting poor shoot production and survival rates (Taylor & Qin, 1993a). This was in part due to the intensive utilization of this species by pandas, particularly between May and June when adult individuals were found to consume as much as 35 kg of bamboo per day (Schaller et al., 1985). Since panda habitat areas located at lower elevations are becoming increasingly fragmented and degraded over time due to human activities (Liu et al., 2001; Xu et al., 2006; Viña et al., 2007), there is potential for localized panda populations to over-utilize stands of *F. robusta* in isolated areas (Hull et al., 2010). However, much more work on this regard needs to be conducted. Regarding the interactions between bamboo and other plant species, the occurrence of overstory tree species influences the occurrence of understory bamboo (Taylor et al., 2004, 2006). Multivariate statistical analyses can be used to identify overstory tree species associated with understory bamboo species. Using the Jaccard similarity index (Jaccard, 1908) combined with hierarchical cluster analyses (i.e., average linkage method), we determined the tree species most likely associated with the six dominant bamboo species in the two field study sites (i.e., three bamboo species in the Qinling Mountains region and

Table 1. Dominant understory bamboo species occurring in the different mountain regions across the entire giant panda range. Mountain regions are indicated in the second column as (1) Qinling; (2) Minshan; (3) Qionglai; (4) Xiangling (both the Greater and Lesser Xiangling); and (5) Liangshan. These bamboo species also constitute the preferred food consumed by pandas. Also shown in the third column are their reported reproductive cycles (i.e., periods between mass flowering events). Names noted in bracketed equivalencies reflect a differing taxonomic usage in the *Flora of China* (Wu et al., 2006).

Species	Mountain region	Reproductive cycle (yrs.)	References
Bashania fargesii (E. G. Camus) Keng f. & T. P. Yi [= *Arundinaria fargesii* E. G. Camus]	1, 2, 3	23	Schaller et al., 1989; Wang, 1989; Tian, 1990; Pan & Lu, 1993; Zhou & Pan, 1997; State Forestry Administration, 2006; Viña et al., 2012
B. faberi (Rendle) T. P. Yi [= *A. faberi* Rendle]	2, 3, 4, 5	45	Campbell & Qin, 1983; Campbell, 1984; Johnson et al., 1988; Reid et al., 1989; Wang, 1989; Tian, 1990; Reid & Hu, 1991; Reid et al., 1991; Taylor & Qin, 1993a; State Forestry Administration, 2006
Chimonobambusa pachystachys Hsueh & T. P. Yi	2, 3, 4	16	Campbell & Qin, 1983; Wang, 1989; State Forestry Administration, 2006
Fargesia scabrida T. P. Yi	2, 3	50	Campbell, 1984; Schaller et al., 1985; Wang, 1989; State Forestry Administration, 2006
F. denudata T. P. Yi	2	40	Schaller et al., 1989; Wang, 1989; State Forestry Administration, 2006
F. dracocephala T. P. Yi	1, 2	n/a	State Forestry Administration, 2006; Li et al., 2007; Viña et al., 2012
F. ferax (Keng) T. P. Yi	4, 5	40	Wang, 1989; State Forestry Administration, 2006
F. nitida (Mitford) Keng f. ex T. P. Yi	1, 2, 3	90	Wang, 1989; Tian, 1990; State Forestry Administration, 2006
F. qinlingensis T. P. Yi & J. X. Shao	1	50	Tian, 1987; State Forestry Administration, 2006; Wang et al., 2007; Viña et al., 2012
F. robusta T. P. Yi	1, 2, 3	37	Campbell & Qin, 1983; Campbell, 1984; Johnson et al., 1988; Reid et al., 1989; Wang, 1989; Tian, 1990; Reid & Hu, 1991; Reid et al., 1991; Pan & Lu, 1993; Zhou & Pan, 1997; State Forestry Administration, 2006
F. rufa T. P. Yi	2	15	Wang, 1989; State Forestry Administration, 2006
Qiongzhuea opienensis (J. R. Xue & T. P. Yi) D. Z. Li & J. R. Xue [= *Chimonobambusa opienensis* (J. R. Xue & T. P. Yi) T. H. Wen & Ohrnberger]	4, 5	20	Wang, 1989; State Forestry Administration, 2006
Yushania chungii (Keng) Z. P. Wang & G. H. Ye [= *Y. brevipaniculata* (Hand.-Mazz.) T. P. Yi]	1, 2, 3	20	Campbell & Qin, 1983; Campbell, 1984; Wang, 1989; State Forestry Administration, 2006
Y. confusa (McClure) Z. P. Wang & G. H. Ye	4, 5	22	Wang, 1989; State Forestry Administration, 2006

three in the Wolong Nature Reserve in the Qionglai Mountains region) (Fig. 4). In both of these regions the species *Betula albosinensis*, a dominant tree species in the giant panda geographic range, was associated with at least three dominant understory bamboo species (Fig. 4). Other species of the genus *Betula* L. (e.g., *B. utilis* D. Don, *B. platyphylla* Sukaczev, *B. luminifera* H. J. P. Winkl.) were also associated with some of the dominant bamboo species (Fig. 4). The occurrence of these species associations can be explained by the interactions between tree species life histories and bamboo life cycles which influence both tree and bamboo regeneration and, thus, contribute to structuring the species composition in the forests of the panda geographic range (Taylor et al., 2004).

People

The pattern of decline of both the distribution and number of pandas has been largely associated with the loss of tree and understory bamboo species, which, in turn, have been attributed to climate fluctuations over many centuries (Schaller et al., 1985; Reid & Gong, 1999; Pan et al., 2001). However, the most recent loss has been attributed to an ever increasing growth of human population and activities (e.g., agricultural expansion, timber harvesting, road construction, livestock grazing, tourism, bamboo harvesting, mining) (Schaller et al., 1985; Reid & Gong, 1999; Pan et al., 2001), together with a drastic increase in the number of households. For example, from 1975 to 2000, the number of local residents in Wolong Nature Reserve increased by 72.4% (from 2560 to 4413) while the number of households jumped by 129.9% (from 421 to 968) (An et al., 2011). The increase in the number of households may have even more drastic negative effects than population growth alone (Liu et al., 2003a), because the proliferation of smaller households (e.g., as a result of divorces and kids moving out of parental homes [An et al., 2003]) translates into more households and less efficient use of natural resources on a per-capita basis (Yu & Liu, 2007).

Human activities vary across space and over time. For example, while cultivation has occurred in the panda geographic range for centuries, it mostly occurred at low elevations which were more accessible to humans (Pan et al., 2001; Loucks et al., 2003). However, in recent decades, cultivation has extended to higher elevations, thus, induced forest clearings where core panda habitat was previously located (Schaller et al., 1985; Wang, 2008). In addition, the extensive timber harvesting and fuel wood collection observed in the panda region between the 1960s and the 1990s were identified to be the most important threats to pandas (Liu et al., 2001; State Forestry Administration, 2006), particularly because clear-cut areas are characterized by two extremes that prevent the occurrence of panda individuals: complete lack of bamboo or extremely dense patches of non-nutritious bamboo without an overstory of trees (Schaller et al., 1985; Bearer et al., 2008).

In addition to changes in the distribution and composition of tree and bamboo species, timber harvesting and fuel wood collection have many other effects that generate feedbacks which ultimately affect local residents. For example, inside Wolong Nature Reserve, timber harvesting occurred along a spatially defined deforestation front in which forests were exploited near households during the 1970s (He et al., 2009). As forests near households depleted, the local residents were forced to cut trees farther away (He et al., 2009). The spatial expansion of timber-extraction activities was also driven by improved accessibility due to the development of road networks. For instance, a project by the United Nations' World Food Programme in the early 1980s designed to mitigate human impacts on panda habitat in the Reserve resulted in a road expansion. This road expansion facilitated the access to a forested area rarely visited during the 1970s, which was subsequently deforested (Wolong Administration Bureau, 2004; He et al., 2009).

Timber harvesting and fuel wood collection not only affect current panda habitat, but also generate legacy effects on future panda habitat (An et al., 2005, 2006; Linderman et al., 2005a) through their conspicuous effects on plant species. For instance, in Wolong Nature Reserve, wood constitutes the main material for traditional house construction. In addition, a significant amount of fuel wood is consumed for cooking, heating, and stewing pig fodder, since pigs are the main animal protein source for local residents (An et al., 2001; Liu et al., 2004). Deciduous tree species such as *Betula albosinensis* and *B. utilis* are preferred for fuel wood, while species such as *Abies faxoniana* are preferred for construction. As mentioned above, these deciduous and evergreen tree species are dominant overstory canopy components in the forests that are suitable for the pandas, but currently they are rarely found in areas near local households, due to excessive harvesting through selective logging. As these species promote the regeneration of understory bamboo suitable for the giant panda (Taylor et al., 2004, 2006), the absence of these species in some areas reduces the likelihood of suitable panda habitat regeneration.

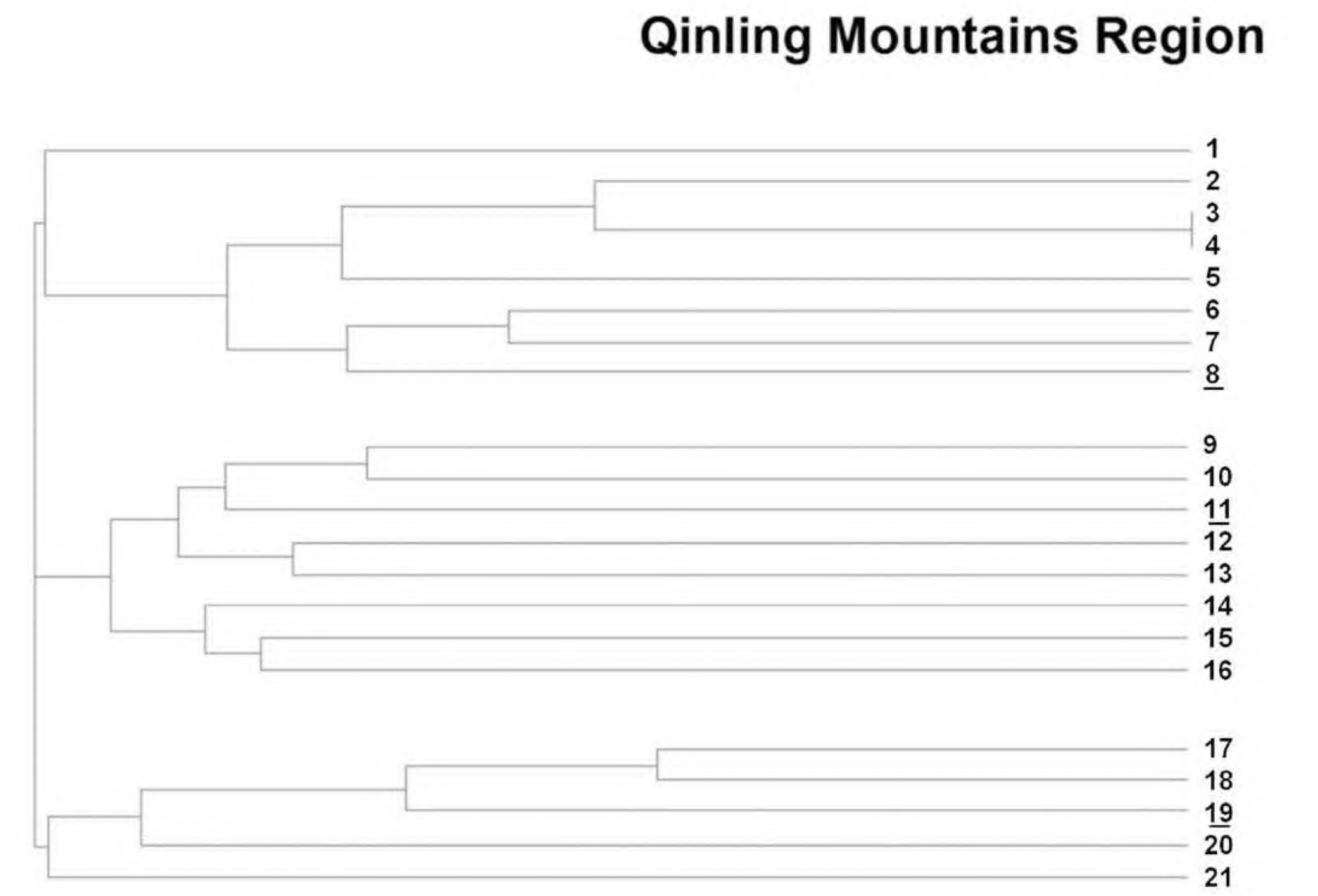


Figure 4. Cluster analysis showing tree and bamboo species associations of six dominant bamboo species (underlined), three of which are located in the Qinling Mountains region and three in the Wolong Nature Reserve located within the Qionglai Mountains region (see Fig. 2 for location). Numbers in the figure correspond to: (1) *Betula platyphylla* Sukaczev; (2) *Quercus spinosa* David ex Franch.; (3) *Ulmus parvifolia* Jacq.; (4) *Pyrus betulifolia* Bunge; (5) *Cornus controversa* Hemsl.; (6) *Quercus liaotungensis* Koidz.; (7) *Betula luminifera* H. J. P. Winkl.; (8) *Fargesia qinlingensis* T. P. Yi & J. X. Shao; (9) *Juglans cathayensis* Dode; (10) *Toxicodendron vernicifluum* (Stokes) F. A. Barkley; (11) *Fargesia dracocephala* T. P. Yi; (12) *Pinus*

Current emerging threats from human activities on pandas and their habitat include the extensive investment in infrastructure (e.g., dams, highways, airports, roads, railroads, tourism facilities) under the West China Development Program. Between 2001 and 2006 alone, this program invested more than U.S. $125 billion for development projects with the main goal of attracting businesses and migrants to western China (Lu, 2009). This construction boom may exacerbate the degradation and fragmentation of giant panda habitat not only in the present but also in the years to come.

An additional threat comes in the form of livestock grazing (Hull et al., 2011). The third panda census reported that livestock grazing is the second most commonly encountered human-driven disturbance, after deforestation (State Forestry Administration, 2006). In the Minshan Mountain region (see its location in Fig. 1), disturbances due to livestock grazing were found in 19% of over 1600 sample plots (Wang, 2008). Despite the recorded prevalence of livestock grazing across the panda geographic range, research on its direct and indirect effects on pandas and their habitat is limited to a handful of case studies (Ran, 2003, 2004; Ran et al., 2003; Kang et al., 2011). These studies mostly reinforced the findings about the prevalence of livestock disturbance in panda habitat areas, hinting that there is some degree of overlap in the habitat selection of pandas and livestock. Therefore, many questions still remain unanswered, particularly regarding whether livestock could threaten the long-term sustainability of understory bamboo, which prior to the increase in livestock grazing was not believed to be threatened by any animal competing with the pandas (Schaller et al., 1985).

Policies

The impacts of human activities on pandas have prompted the Chinese government to develop a series of policies, including the establishment of panda nature reserves (protected areas specifically designed to conserve the panda). A total of 63 nature reserves (by 2007) have been designated to conserve the habitat for the panda. While these panda reserves contain about 40% of the current panda habitat (Viña et al., 2010), they tend to be isolated (Viña et al., 2007, 2010), which means that there is still a significant amount of panda habitat outside the nature reserve system. However, nature reserves have not been immune to human threats, as the panda habitat contained within their boundaries also experienced a steady decrease (Liu et al., 2001; Viña et al., 2007). This has occurred despite the fact that these reserves have imposed restrictions on resource use (e.g., fuel wood) (He et al., 2009) and are required to establish zoning designations to spatially contain human activities (Hull et al., 2011). In many cases these designations are ineffectual because the boundaries of zoning designations are displaced in response to development pressures (e.g., construction of tourism facilities) (Jim & Xu, 2004; Liu & Li, 2008; Hull et al., 2011). In addition, panda reserves often lack sufficient funding for their operations (Liu et al., 2003b) and, thus, do not properly enforce conservation activities (Lü & Kemf, 2001).

In addition, since the late 1990s the Chinese government has been implementing two of the largest ecological conservation programs in the world: (1) The Natural Forest Conservation Program (NFCP), which bans logging in natural forests in order to prevent illegal harvesting (Yang et al., 2013b), and (2) the Grain-to-Green Program (GTGP; also referred to as the Sloping Land Conversion Program), which encourages farmers to return steep cropland to forest or grassland (Uchida et al., 2005; Liu et al., 2008). Both of these policies have global implications, as they fulfill part of China's commitment to international biodiversity conservation treaties (Liu et al., 2008). These policies seem to have been producing overall positive effects on forests, including those in panda habitat regions (Li et al., 2013), by preventing further deforestation and promoting forest recovery (Viña et al., 2007, 2011; Liu et al., 2008, 2013). The implementation of the NFCP has started to also exhibit a significant positive effect on panda habitat, particularly when local people are actively involved in forest monitoring activities (Tuanmu, 2012). This is because timber harvesting has been curbed since

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armandii Franch.; (13) *Quercus aliena* Blume; (14) *Betula albosinensis* Burkill; (15) *Prunus* L. sp.; (16) *Acer davidii* Franch.; (17) *Betula albosinensis* var. *septantrionalis* C. K. Schneid.; (18) *Abies fargesii* Franch.; (19) *Bashania fargesii* (E. G. Camus) Keng f. & T. P. Yi [= *Arundinaria fargesii* E. G. Camus]; (20) *Larix chinensis* Beissn.; (21) *Euonymus phellomanus* Loes.; (22) *Abies faxoniana* Rehder & E. H. Wilson; (23) *Fargesia nitida* (Mitford) Keng f. ex T. P. Yi; (24) *Betula utilis* D. Don; (25) *Prunus dielsiana* (C. K. Schneid.) Koehne; (26) *Quercus aquifolioides* Rehder & E. H. Wilson; (27) *Salix dissa* C. K. Schneid.; (28) *Salix phanera* C. K. Schneid.; (29) *Tilia intonsa* E. H. Wilson; (30) *Acer laxiflorum* Pax; (31) *Corylus ferox* Wall. var. *thibetica* (Batalin) Franch.; (32) *Fargesia robusta* T. P. Yi; (33) *Rhododendron pachytrichum* Franch.; (34) *Tsuga chinensis* (Franch.) E. Pritz.; (35) *Cotoneaster moupinensis* Franch.; (36) *Sorbaria arborea* C. K. Schneid.; (37) *Salix dolia* C. K. Schneid.; (38) *Syringa* L. sp.; (39) *Bashania faberi* (Rendle) T. P. Yi [= *Arundinaria faberi* Rendle]; (40) *Prunus pilosiuscula* (C. K. Schneid.) Koehne.

the late 1990s due, in part, to the national logging ban implemented through the NFCP (State Forestry Administration, 2006; Viña et al., 2007, 2011; Li et al., 2013). This ban has brought not only a reduction in the rate of loss of natural forests, but also forest regeneration in previously clear-cut areas (Viña et al., 2011; Li et al., 2013). Such forest regeneration brings some hope for panda conservation, since pandas have been observed to use secondary forests that are 30+ years old if adequate understory bamboo is present (Viña et al., 2007; Bearer et al., 2008).

Nationwide, the GTGP has converted 8.8 million ha of cropland into forest or grassland (Liu et al., 2013b). In the Qionglai Mountains region, this program provides seedlings for ca. 48 tree species, many of which are exotic (Viña et al., 2013) and do not constitute suitable tree species that promote bamboo regeneration, nor giant panda habitat recovery. Among the species planted under the GTGP, *Cunninghamia lanceolata* (Lamb.) Hook., *Magnolia officinalis* Rehder & E. H. Wilson, *Ligustrum lucidum* W. T. Aiton, *Cryptomeria japonica* (Thunb. ex L. f.) D. Don, *Eucommia ulmoides* Oliv., and *Alnus cremastogyne* Burkill are the species used more often (Viña et al., 2013). The Wolong Nature Reserve has, in addition to the GTGP (Chen et al., 2009a, 2009b, 2010, 2012b), a local Grain-to-Green/Bamboo Program (GTGB) in which farmers are compensated for actively planting the species *Fargesia robusta* in previous cropland areas (Yang et al., 2013a, 2013b). The planted bamboo is harvested particularly to provide fodder for the captive pandas in the breeding center of the Reserve. As these monospecific bamboo plantations generally lack overstory trees, they could not be considered suitable habitat areas for the pandas, either now or in the foreseeable future. Thus, promoting the re-growth of tree species that characterize suitable panda habitat should be one of the priorities in the panda conservation toolbox.

Macroscale Factors

Plants and pandas are not only affected by local factors, but also by large-scale factors such as migration, tourism, natural disasters, and climate change. Since China has been the fastest-growing economy in the world over the past three decades (Liu & Diamond, 2008), there has been an increase in job opportunities in cities; thus, more rural people are engaging in labor migration. This has the effect of reducing deforestation (e.g., through a reduction in fuel wood demands) while enhancing the natural regeneration of forests, particularly in giant panda regions (Chen et al., 2012a). In addition, many nature reserves in China are exhibiting a rapid increase in the influx of tourists from around the world (Han, 2000; Liu et al., 2003a). The increase in tourism is supported by rapid development of tourism facilities (e.g., hotels, restaurants), together with expansions in the road network, all of which influence the abundance and distribution of plants and pandas. For example, the number of tourists visiting Wolong Nature Reserve increased dramatically, from ca. 20,000 in 1995 to ca. 100,000 in 2000 (Lindberg et al., 2003) and to more than 200,000 in 2006 (He et al., 2008). During this time period, the number of households participating in tourism activities also increased, yet households with more livelihood assets (e.g., income, education, social capital) were more likely to participate than less affluent households (Liu et al., 2012).

Another macroscale factor is represented by natural disasters. As in most mountain regions around the world, landslides are the most common natural disaster in the panda geographic range (Brabb & Harrod, 1989; Lee, 2005; Pradhan et al., 2006). More than 70% of catastrophic landslides are related to land use change (Reid et al., 2006; Huang, 2007; Rindfuss et al., 2008) and are triggered by severe rainfall events during the summer months (Li, 1989), and by a high seismic activity, with four earthquakes larger than 7.0 on the Richter scale occurring in the region during the last hundred years (Wang et al., 2008). The last such earthquake was the 7.9 M_w (8.0 Richter scale) 12 May 2008 earthquake, the epicenter of which was located in Wenchuan County, Sichuan Province. Earthquake-induced landslides not only affect many forest areas (Viña et al., 2011) and giant panda habitat (Wang et al., 2008; Xu et al., 2009) (Fig. 5), but also induce significant losses to tree and shrub species richness (Zhang et al., 2011). Earthquake-induced landslides are so severe in many areas that they offset the gains in forest cover obtained through the implementation of conservation policies such as the NFCP (Viña et al., 2011).

A final macroscale factor potentially affecting plants and pandas is human-induced climate change. While deforestation and forest degradation are threatening the survival of about half of all bamboo species worldwide (Bystriakova & Kapos, 2006), climate change may present an additional significant threat. Many bamboo species are vulnerable to climate change because their unusual extended sexual reproduction intervals (from 10 to 120 years) (Janzen, 1976) and limited seed dispersal ability (Taylor et al., 1991) render them less capable of adjusting their distributions to the rapidly changing climate projected to occur within this century (IPCC,

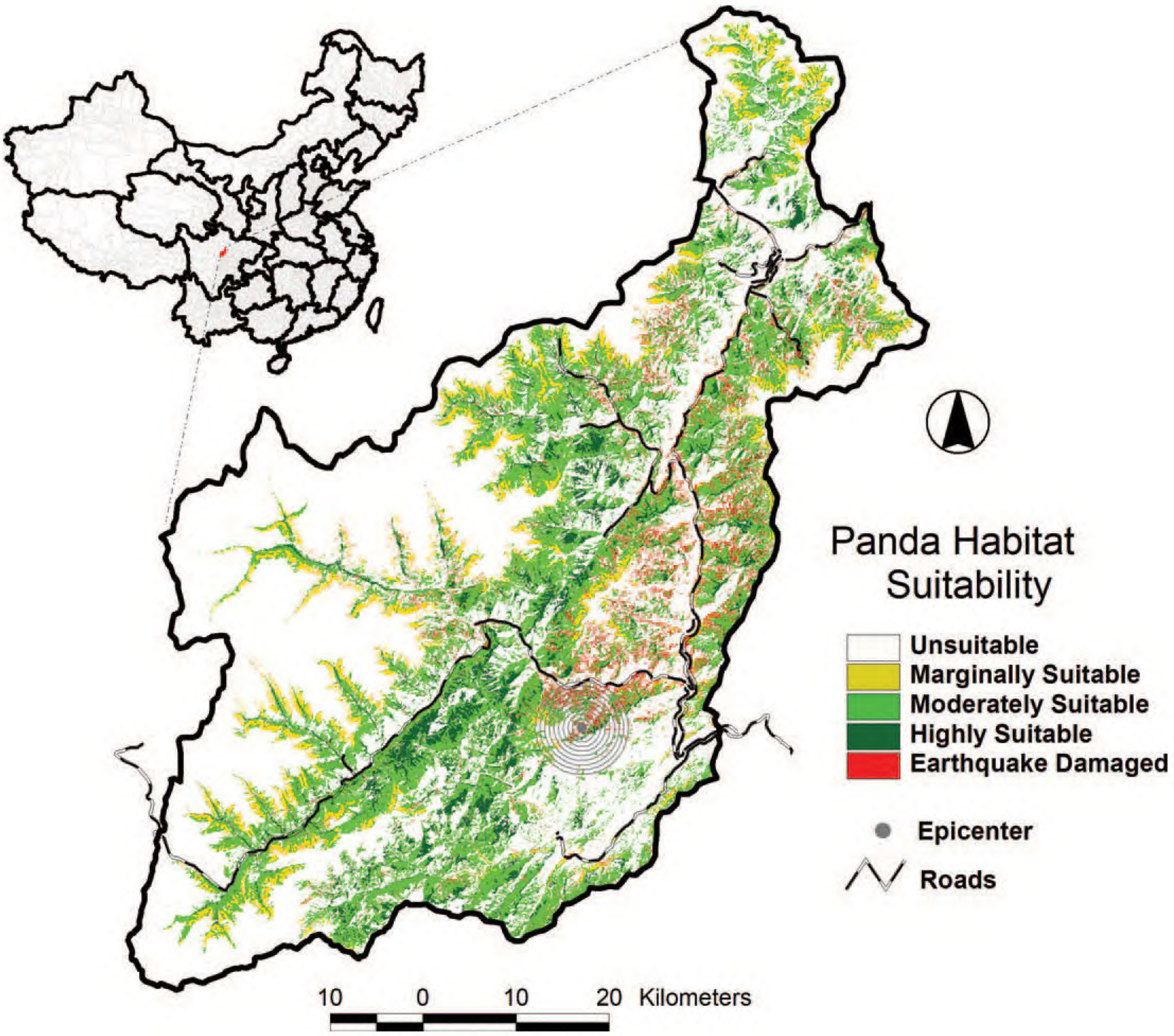


Figure 5. Spatial distribution of panda habitat areas (modified from Liu et al., 2001; Viña et al., 2007) in Wenchuan County damaged by the 12 May 2008, Wenchuan Earthquake. Wolong and Caopo nature reserves are located entirely within Wenchuan County.

2007). In addition, bamboo species have limited vegetative dispersal ability (e.g., ca. 0.2–0.35 m/year for *Bashania fargesii* and *Fargesia robusta*) (Tian, 1989; Taylor & Qin, 1993a). Yet, despite this vulnerability of bamboo species to climate change, few studies have evaluated the potential effects of climate change on bamboo distribution and how these will cascade to pandas. A recent ensemble of bamboo distribution projections associated with multiple climate change projections and bamboo dispersal scenarios indicates that a substantial reduction in the distributional ranges of the three dominant bamboo species in the Qinling Mountains of China may occur by the end of the 21st century, with potentially drastic effects in the distribution of suitable giant panda habitat (Tuanmu et al., 2013). For instance, two contrasting climate change projections derived from two general circulation models (one developed by the Commonwealth Scientific and Industrial Research Organisation, Australia [CSIRO-Mk2] and the other from a coupled model developed by the Center for Climate System Research and the National Institute for Environmental Studies, Japan [CCSR/NIES]) under the conservative B2 greenhouse gas emission scenario (which emphasizes local solutions to economic, social, and environmental sustainability) (IPCC, 2007), project reductions and distributional shifts of climatically suitable areas (CSA) of the three bamboo species by the end of the century (i.e., 2070–2099). Under the CSIRO-Mk2, there is a conspicuous projected shift of the CSA of the three species toward higher latitudes as compared with current conditions (Tuanmu et al., 2013) (Fig. 6). Under the CCSR/NIES, the CSA of *F. qinlingensis* is projected to disappear, as this species currently occurs at higher elevations, while the CSA of *B. fargesii* and *F.*

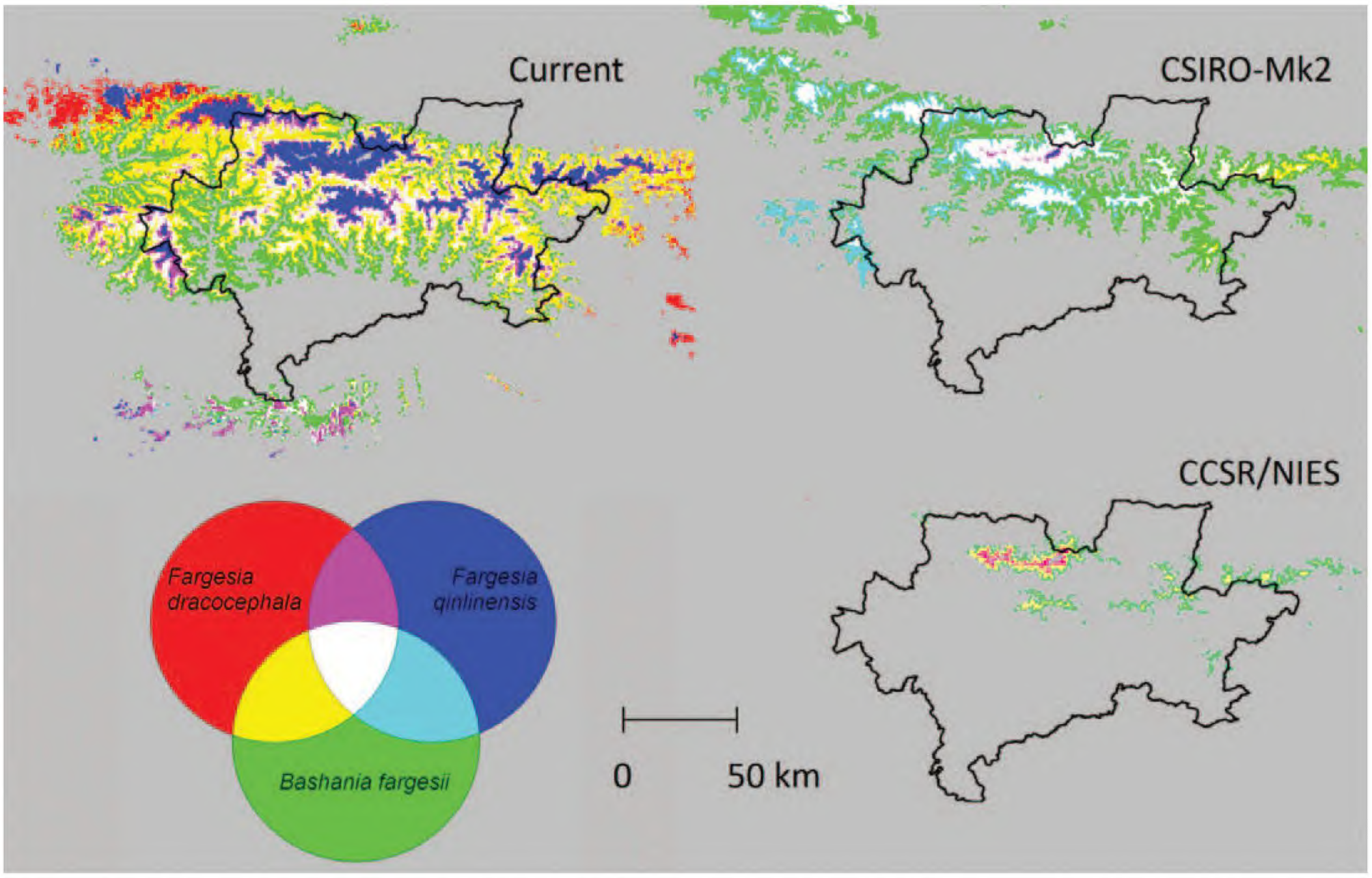


Figure 6. Current and projected (2070–2099) distributions of climatically suitable areas for the three dominant bamboo species in the Qinling Mountains region of China (after Tuanmu et al., 2013): *Fargesia dracocephala* (red), *F. qinlingensis* (blue), and *Bashania fargesii* (green). Their co-occurrences are indicated by the overlapping colors of the three species circles. The climate projections were derived from two global circulation models (one developed by the Commonwealth Scientific and Industrial Research Organisation, Australia [CSIRO-Mk2], and the other a coupled model developed by the Center for Climate System Research and the National Institute for Environmental Studies, Japan [CCSR/NIES]) under the B2 scenario of the IPCC Special Report on Emission Scenarios (IPCC-SRES). The black line corresponds to the political boundary of the Qinling Mountains region in Shaanxi Province, China. For its location within the panda geographic range, see Figure 2.

dracocephala are projected to experience a substantial reduction (Tuanmu et al., 2013) (Fig. 6). However, irrespective of the global circulation model used, the projected location of the CSA is distant from current bamboo distribution ranges, which may hinder potential range shifts of bamboo species (as well as pandas) in response to the projected change in the global climate (Tuanmu et al., 2013). This is particularly exacerbated by the long history of human activities surrounding the Qinling Mountains, which are not projected to substantially reduce in the foreseeable future.

Conclusions

The current pace of biodiversity loss worldwide is intensifying the need for conservation actions that address not only species of conservation interest, but also their interactions with multiple other species, as well as with humans. Using the CHANS framework, this article has illustrated the complex relationships among pandas, plants (tree and bamboo species), and humans. Our synthesis indicates that many human factors, including socioeconomic and demographic, exhibit reciprocal interactions with pandas and the plant species that comprise their habitat. The framework is particularly useful for uncovering key patterns and processes behind plant–animal interactions modified by human activities. Understanding of such patterns and processes is crucial for developing and evaluating policies that better reconcile the needs of an ever-growing human population with biodiversity conservation.

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ORGANISMIC REMOTE SENSING FOR TROPICAL FOREST ECOLOGY AND CONSERVATION[1,2]

Gregory P. Asner[3]

Abstract

Tropical forests are vast and scientifically underexplored places. Biotic losses, gains, and reorganization within these systems go undetected due to a lack of access to technologies needed to monitor forest cover, composition, and carbon content. Provision of forest cover-monitoring tools for non-scientists has, thus, become a focus of innovation in the remote-sensing community, while advances in high-resolution forest carbon and biodiversity mapping science has progressed more slowly. This paper focuses on high-resolution remote-sensing developments to measure and monitor tropical forest canopies at the "organismic scale," which is the resolution that resolves individual canopies and species throughout the forest landscape. Emphasis is placed on how forest carbon stocks can be mapped with precision and accuracy comparable to that of field-based estimates. Biodiversity mapping poses the greatest challenge, but recent advances in three-dimensional functional and structural trait imaging can reveal variation in species richness, abundance, and functional diversity over large geographic regions. The pay-off in pursuing these studies will be a vastly improved understanding of tropical biodiversity patterns and their underlying ecological and evolutionary drivers, which will have positive cascading effects on conservation decision-making and resource policy development.

Key words: Biodiversity, canopy chemistry, Carnegie Airborne Observatory (CAO), conservation, functional diversity, LiDAR, rainforest, tropical forest.

Introduction

Following four billion years of evolution on Earth, the most biologically diverse biome to have arisen on land is the great swath of tropical forest straddling the equator. However, tropical forests continue to undergo losses and biotic rearrangement in complex spatial patterns and at variable rates that defy most conservation policy initiatives (Venter et al., 2013; Lindenmayer et al., 2014). Despite the importance of the tropics, a plethora of barriers exist to the effective monitoring of changes in forest cover and composition at large enough geographic scales, and with the required spatial detail, to create transparent infor-

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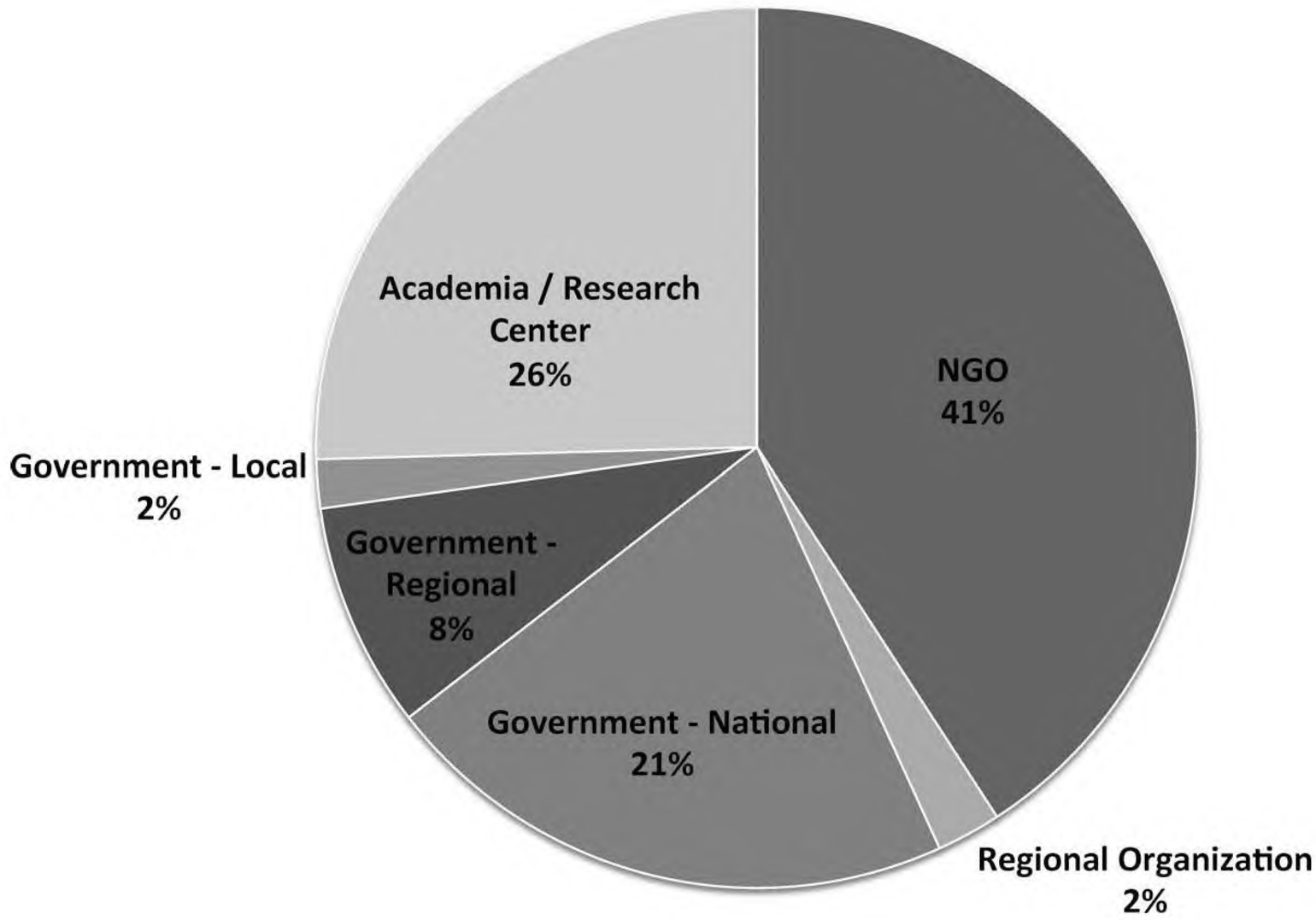


Figure 1. The distribution of the CLASlite deforestation and forest degradation mapping community in Latin America (among 1700 organizations as of August 2014). NGO is a non-government organization within any given country. A regional organization is one that operates in two or more countries.

mation for the management and conservation of this exceptional biome. What are the barriers, and how do we break them down?

In terms of forest cover monitoring, including deforestation and forest degradation, a persistent problem has been the inaccessibility of straightforward satellite-based technology. For years, the Brazilian Institute for Space Research has led the way toward removing this barrier, with a state-of-the-art deforestation monitoring system for the Brazilian Amazon (Instituto Nacional de Pesquisas Espaciais [National Institute for Space Research; INPE], 2007). Now a parallel system operated by the Brazilian non-government organization IMAZON (<http://imazon.org.br>) monitors both deforestation and forest degradation. Several other monitoring systems are also operated by experts (reviewed by Achard et al., 2007), providing national to global-scale estimates of deforestation, albeit with degradation rarely included (Global Observation of Forest and Land Cover Dynamics [GOFC-GOLD], 2008). In many cases, the forest change maps are being actively disseminated, and these efforts demonstrate that vast regions of tropical forest cover can be monitored. However, a growing number of stakeholders want to conduct their own monitoring, so providing access to the technology has recently become a focus of innovation in the mapping sciences.

To address this challenge, some groups are making the techniques, software, and support available for non-experts wishing to monitor forest cover. The Carnegie Institution's system, called CLASlite, was designed to provide the tools for monitoring deforestation and forest degradation directly to the end-user (Asner et al., 2009b). CLASlite is based on the original Carnegie Landsat Analysis System (CLAS; Asner et al., 2005, 2009d; Oliveira et al., 2007; Broadbent et al., 2008), and it has been used to map changes in forest cover and disturbance in various regions of the world (e.g., Carlson et al., 2012; Allnutt et al., 2013; Carvalho et al., 2013). Supporting input from eight different satellites, CLASlite also helps users overcome some, but not all, problems of access to data derived from otherwise hard-to-use satellite imagery. The system runs on a standard personal computer and has been actively disseminated to users in more than 1700 organizations in over 120 countries throughout the world (Fig. 1; <http://claslite.ciw.edu>). Deforestation Alert System (Sistema de Alerta de Desmatamento; <http://imazon.org.br>) and other systems (Hansen et al., 2013) are also coming online via Google's Earth Engine (<http://earthengine.google.com>), which is giving users around the world access to free forest cover-monitoring tools. These and other initiatives suggest that monitoring forest cover is both technically tractable and on the rise institutionally.

Despite these advances in monitoring tropical forest cover, it is clear that we must also continue to seek ways to map what lies within tropical forests, including the canopy, carbon content, and biodiversity in terms of species presence, abundance, richness, and their functional traits. The need for

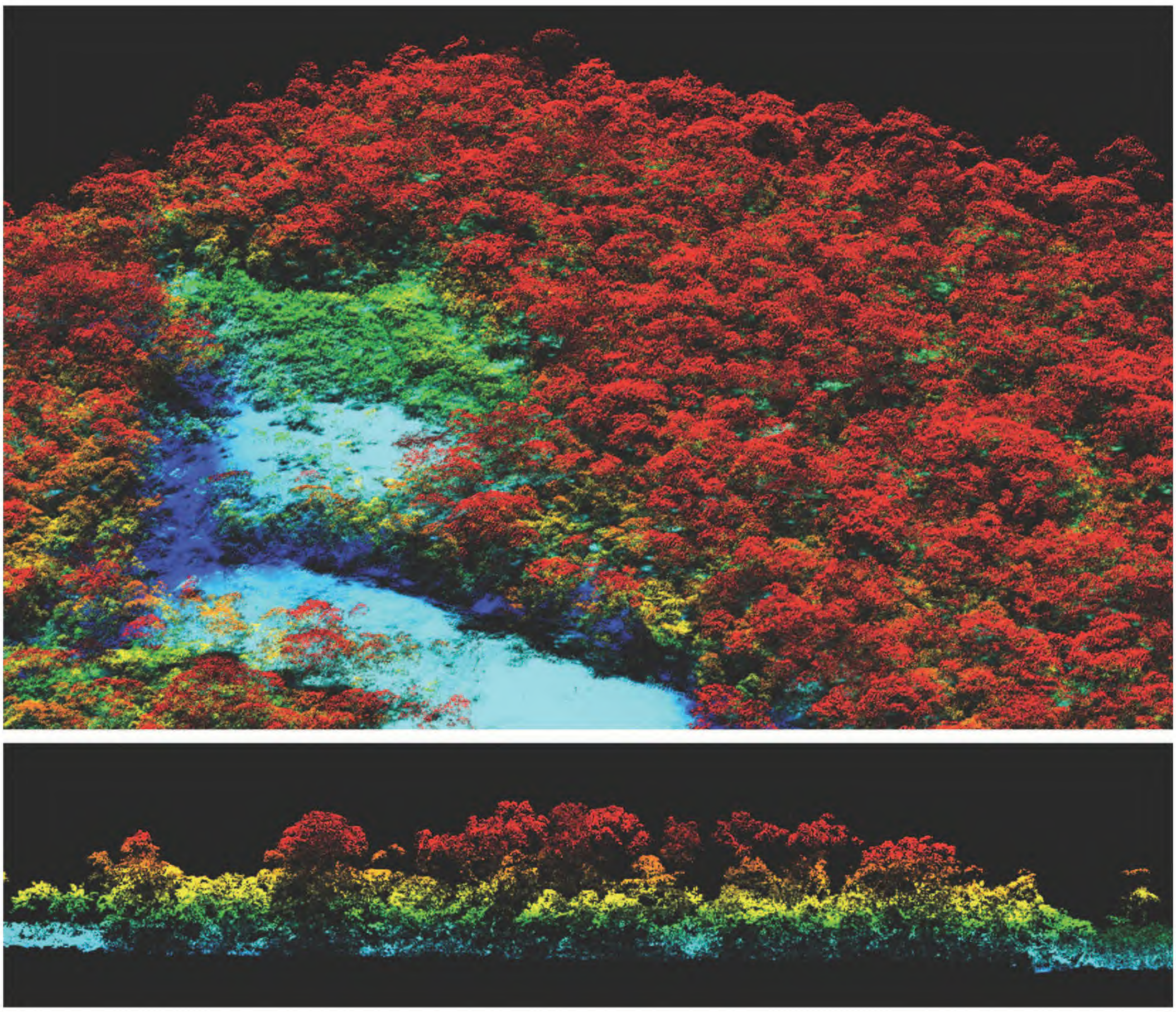


Figure 2. Airborne Light Detection and Ranging (LiDAR) provides three-dimensional imaging of ecosystems by measuring precise distances between ground and vegetation elements including foliage and woody stems. LiDAR works by sweeping a high-pulse rate laser outward from the bottom of an airplane, recording the elapsed time between laser pulses and returns. The upper image of the lowland Amazonian forest in Colombia was collected by the Carnegie Airborne Observatory (CAO; <http://cao.ciw.edu>) and is color-coded by height above ground as shown in the lower sectional view. Red indicates taller portions of the canopy; blue indicates shorter portions of the canopy.

tropical forest carbon monitoring has arisen throughout the conservation and climate-change communities, particularly as voluntary markets have provided project-level funds to protect forests based on carbon offsets (Parker et al., 2009) and as policy development continues toward compliance carbon-offset regulations at international (United Nations Framework Convention on Climate Change [UNFCCC], 2009) and jurisdictional (Odigha, 2010) levels.

Tropical forest carbon stocks also express the outcome of myriad evolutionary, ecological, and environmental processes, and, thus, carbon mapping could enrich our understanding of these processes operating at multiple spatial and temporal scales. Meanwhile, biodiversity monitoring continues to grow in importance as scientists, conservationists, and decision-makers face the specter of land use and climate-induced shifts and losses of species and communities (Jetz et al., 2007; Williams et al., 2007; Hubbell et al., 2008; Malhi et al., 2009; Asner et al., 2010a). Yet, spatially explicit, high-resolution maps of carbon stocks and biodiversity rarely exist, and certainly not over extensive reaches of tropical forest. Why is that the case? High-resolution mapping science is only now figuring out how to get at the required measurements remotely, and some of the advances are too new for, or simply do not work with, more widely available satellite instrumentation (Schimel et al., 2013). In the remainder of this paper, I focus on some advances in mapping of tropical forest carbon stocks and composition at the "organismic

scale," that is, with detail resolving individual canopies and species throughout the forest landscape.

Forest Carbon

Maps of tropical forest carbon stocks have traditionally relied upon field inventory plots, in which stem diameters and estimates of canopy height are measured and recorded by hand (USDA Forest Service, 2008). The problem posed by this traditional inventory approach is that it requires a dense array of plots in order to characterize different types of forest, forest conditions including different ages of secondary regrowth, forests of varying levels of degradation, and environmental controls over spatial patterns in carbon stocks. The number of plots required to meet this need is often dizzying from both logistical and financial standpoints. Additional resources are, therefore, needed to decrease the reliance on field plots and to provide spatially contiguous data that will support initiatives to reduce carbon emissions from tropical forests.

Developing high-resolution carbon maps requires measuring forest stocks as accurately as field plots, while also sampling over large, continuous gradients of climate, topography, hydrology, soils, and biological diversity. The measurements must also resolve highly variable effects of land use on carbon stocks. A major new contributor to solving the carbon mapping challenge is airborne Light Detection and Ranging (LiDAR), a technology that images forest canopies in three dimensions using emitted laser light pulses (Lefsky et al., 2002b; Mascaro et al. 2014) (Fig. 2). When calibrated to on-the-ground carbon stocks, LiDAR has provided high-resolution, spatially contiguous estimates of aboveground carbon throughout tropical landscapes (Drake et al., 2002a, 2002b; Lefsky et al., 2002a; Clark et al., 2011).

Airborne LiDAR-derived maps of tropical forest carbon have proven increasingly precise and accurate, with errors comparable to those derived from field plots (Mascaro et al., 2011b). Tropical forest field plots based on traditional allometric equations produce errors in the 10%–25% range (Keller et al., 2001; Chave et al., 2004; Alves et al., 2010); the prediction errors inherent in using LiDAR are now well within that range, hovering around 10%–13% compared to estimates based on exhaustively measured field plots (Asner et al., 2012c; Zolkos et al., 2013). Although LiDAR can be expensive to use over small project areas, there is a large economy-of-scale effect that can reduce its cost to less than US 10¢ per hectare in larger projects (Asner et al., 2010b, 2012a). As plot-based estimates continue to improve, LiDAR-based estimates will also advance to greatly extend the limited spatial density and geographic extent of tropical plot networks.

Research in the airborne LiDAR arena has demonstrated the tractability of tropical forest carbon mapping at large geographic scales of thousands to millions of hectares. High-resolution LiDAR-based maps are particularly useful because other field- and map-based ecological data can be integrated with them. This has afforded the opportunity to study how environmental and land-use factors influence forest structure and carbon stocks, and how these controls may be tied to gradients in biological diversity. For example, Mascaro et al. (2011a) found that topographic slope was positively correlated with aboveground carbon stocks on Barro Colorado Island in the Republic of Panama. Based on other studies, the LiDAR findings also suggest that erosional surfaces on or at the foot of hillslopes, which often harbor soils of locally higher fertility compared to topographic crest areas (Porder et al., 2005; John et al., 2007) and which can be associated with unique species compositions in some forests (Clark et al., 1999), may result in elevated carbon stocks. Combining LiDAR and hyperspectral remote-sensing data in Hawaiian rainforests, Asner et al. (2009a) developed a similar set of topographically mediated results but also resolved the effects of species on carbon storage. Carbon was found to vary with slope, elevation, and soil age, and the imprint of species on carbon stocks trumped climate and fertility gradients by up to 300%. This approach, combined with satellite data on forest cover and deforestation, subsequently provided a means to map the effects of environment, humans, and other species on carbon stocks throughout Hawai'i (Asner et al., 2011b) (Fig. 3). These and other studies reveal underlying controls on forest carbon, indicating functional processes as well as impacts of human activities.

Canopy Diversity

While high-resolution forest carbon monitoring has advanced at a rapid pace, parallel undertakings with biodiversity mapping have proven more challenging. Rather than focusing on the better-established role of remote sensing in determining broad-scale aspects of biodiversity, such as vegetation patch size, shape, and location, among others (Innes & Koch, 1998; Foody, 2003; Turner et al., 2003; Gillespie et al., 2008), here, the emphasis is on the richness and abundance of plant species and functional types within tropical forests. The focus is specifically related to the organismic scale that can discern individuals within the canopy, although some very innovative work linking medium-resolution (~30 m)

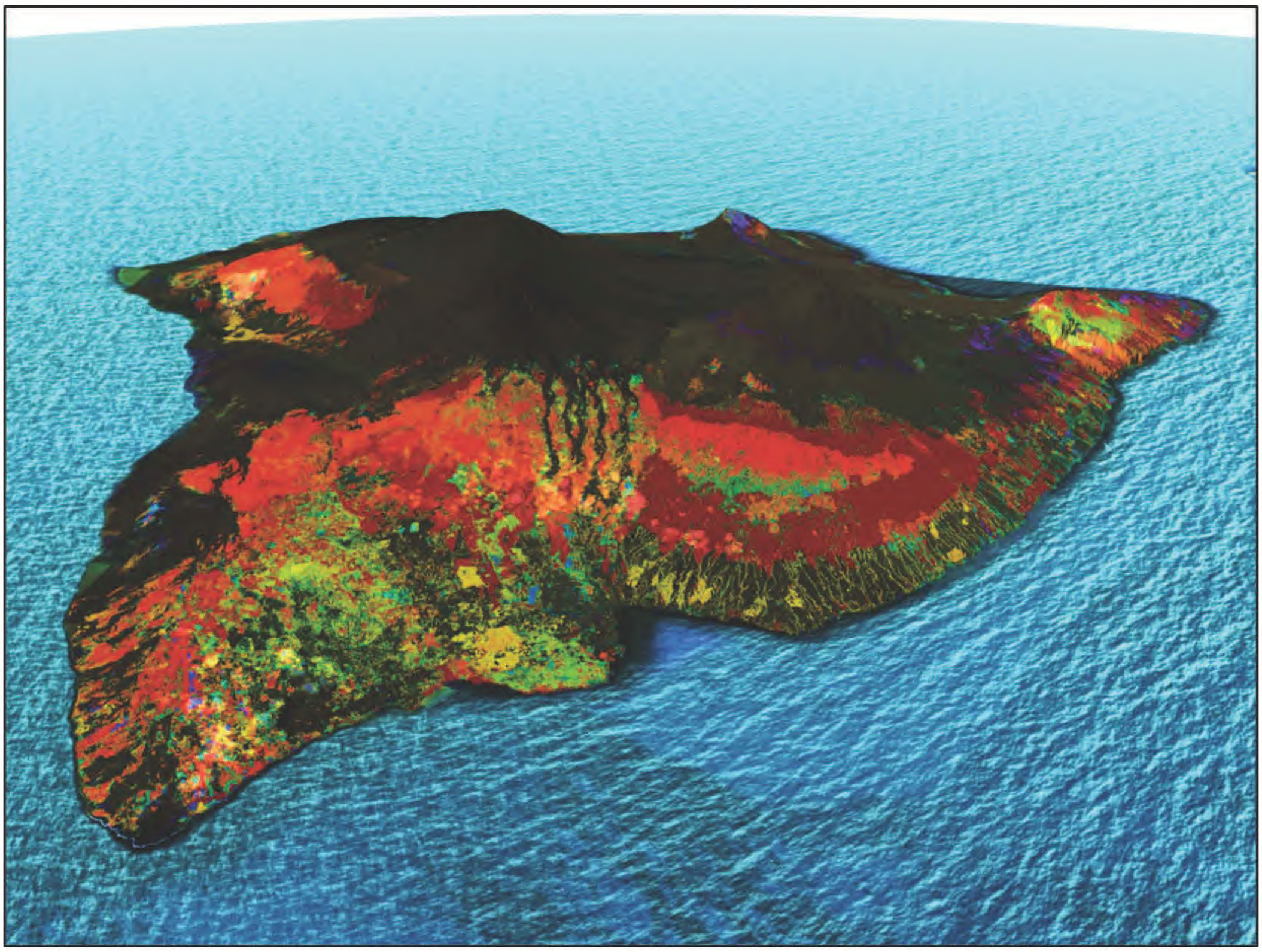


Figure 3. A first-time view of aboveground vegetation carbon stocks across the Island of Hawai'i, derived from airborne LiDAR and satellite data, draped over a digital elevation model. The regional distribution of carbon stocks shows high values in red (> 100 Mg C ha^{-1}) corresponding to humid tropical forests and plantations, and lows in purple (< 20 Mg C ha^{-1}) representing dry woodland vegetation. This map was created from data provided by Asner et al. (2011).

satellite metrics with biodiversity gradients should not be forgotten (Saatchi et al., 2008; Gillespie et al., 2009; Rocchini et al., 2010). Much of the work described in this section requires airborne data, although high-resolution (1–4 m) spaceborne images are also contributing to the effort (Hurtt et al., 2003; Read et al., 2003; Clark et al., 2004a; Palace et al., 2008).

The portion of forest diversity most accessible to organismic remote sensing is the canopy, and, thus, this is where mapping studies have naturally focused. Given the central role that canopies play in harboring all flora and fauna (Connell, 1978; Orians et al., 1996) and in mediating millions of interactions among the biota (Janzen, 1970), there is no doubt that mapping canopy diversity will transform the way that tropical ecosystems are studied. Clearly, mapping would also have a positive impact on tropical forest assessments used in conservation planning and policy development, since it would identify the location and extent of biodiversity gradients and hotspots.

The challenge with canopy-diversity mapping ultimately rests in identifying plant traits that indicate spatial variability among organisms and which of those traits can also be detected via remote sensing. Plant traits not only express variation in ecological processes (Wright et al., 2004; Ollinger, 2011), but also can be related to variation in species composition and phylogeny (Rees et al., 2001; Funk et al., 2008; Ackerly, 2009). With respect to remote sensing, the potential traits encompass functional characteristics, such as spectral and chemical properties, as well as canopy structural characteristics (Table 1). Spectral traits are those describing the reflectance, transmittance, and absorptance patterns at leaf and canopy levels. Chemical traits include a wide variety of compounds mediating light capture and growth, longevity, and defense (Asner et al., 2011c). Light capture-growth traits include photosynthetic pigments, such as chlorophylls and carotenoids, nitrogen, and phosphorus (Evans, 1989; Niinemets et al., 1999). Longevity and defense compounds include lignin and cellulose that are

Table 1. Some plant traits that have proven to be related to biological diversity and which are also accessible via remote sensing. Example references are provided, with an emphasis on tropical forests where possible.

Trait	Example references
Functional	
Spectral reflectance	Carlson et al. (2007), Ustin & Gamon (2010)
Light absorption	Avalos et al. (1999), Lucas & Carter (2008)
Chlorophylls	Castro-Esau et al. (2006), Sanchez-Azofeifa et al. (2009)
Carotenoids-anthocyanins	Blackburn (1998), Sims & Gamon (2002)
Leaf mass per area (LMA)	Poorter (2000), Asner et al. (2011e)
Nitrogen and phosphorus	Porder et al. (2005), Martin et al. (2008)
Base cations (Ca, Mg, K)	Asner & Martin (2011), Asner et al. (2011d)
Lignin, cellulose	Curran et al. (1997), Almeida & Filho (2004)
Phenols, tannins	Kursar et al. (2009), Skidmore et al. (2010)
Water	Asner et al. (2004), Clark et al. (2011)
Structural (canopy)	
Leaf area index (LAI)	Thenkabail et al. (2004), Gillespie et al. (2009)
Height	Drake et al. (2002b)
Crown diameter	Read et al. (2003), Clark et al. (2004a)
Crown depth	Clark et al. (2004b)
Vertical foliage profile	Weishampel et al. (2000), Asner et al. (2008a)

structurally tough (Melillo et al., 1982) as well as phenols and tannins that can be used against pests and pathogens (Coley et al., 1993). An example of an overarching or synthetic structural trait is leaf mass per area (LMA). Although not directly indicative of a chemical compound or element, LMA often correlates with growth and longevity chemistries (Wright et al., 2004; Poorter et al., 2009), and LMA has a strong role in determining the spectral reflectance properties of tropical canopies (Asner et al., 2011e). Canopy structural traits range from leaf area index (LAI), which is the total projected leaf area per unit ground area (Kira et al., 1969), to canopy height, crown diameter and depth, and the vertical distribution of foliage (Lefsky et al., 2002b).

Progress in canopy-diversity mapping has been made on several fronts, at times based explicitly upon the detection and interpretation of these plant functional and structural traits. Work in this arena can be partitioned into species richness, species detection, and functional diversity. Canopy richness is defined as the number of canopy species per unit area. From a remote-sensing perspective, richness mapping has not necessarily required that each species be identified. Instead the number of unique organisms per unit area can be detected based on spatial variation in traits. Studies show that this can be achieved either by directly associating spectral diversity with species richness, or by converting remotely sensed metrics to plant traits followed by classification of these traits to diversity estimates. A number of satellite studies have related optical and radar signals with spatial gradients in species richness (e.g., Foody & Cutler, 2003; Saatchi et al., 2008; Rocchini et al., 2010). However, at organismic scales, Carlson et al. (2007) first found that spectral diversity varies with species richness per unit area in lowland Hawaiian tropical forests (Fig. 4). Their work, like that of many others in this particular research area, utilized airborne imaging spectroscopy, which is composed of precisely co-located images in hundreds of narrow and contiguous spectral channels often spanning the 400–1050 nm (visible to near-infrared) or 400–2500 nm (visible to shortwave-infrared) wavelength range (Goetz et al., 1985; Asner et al., 2012d). A reflectance signal derived for each spectrometer image pixel then allows for detailed analysis using a wide variety of canopy spectral and chemical traits, summarized by Blackburn (2007), Kokaly et al. (2009), Ustin et al. (2009), and many others.

With imaging spectrometer data, Carlson et al. (2007) estimated species richness for very tightly packed canopies of up to 33 trees per 0.1 ha and found that richness levels were elevated in zones where native and invasive species intermix in communal areas outside of the few remaining protected areas that harbor lower diversity communities harboring highly endemic species. This type of airborne work has been mirrored in leaf-level studies of tropical forests, where increasing spectral diversity has been linked to biological diversity among canopy species in Panama (Castro-Esau et al., 2006) and elsewhere in Hawai‘i (Féret & Asner, 2011). Critically, these and other studies (e.g., Sanchez-Azofeifa et al., 2009) demonstrate that spectral diversity is a direct expression of leaf chemical diversity, particularly among the chemical elements

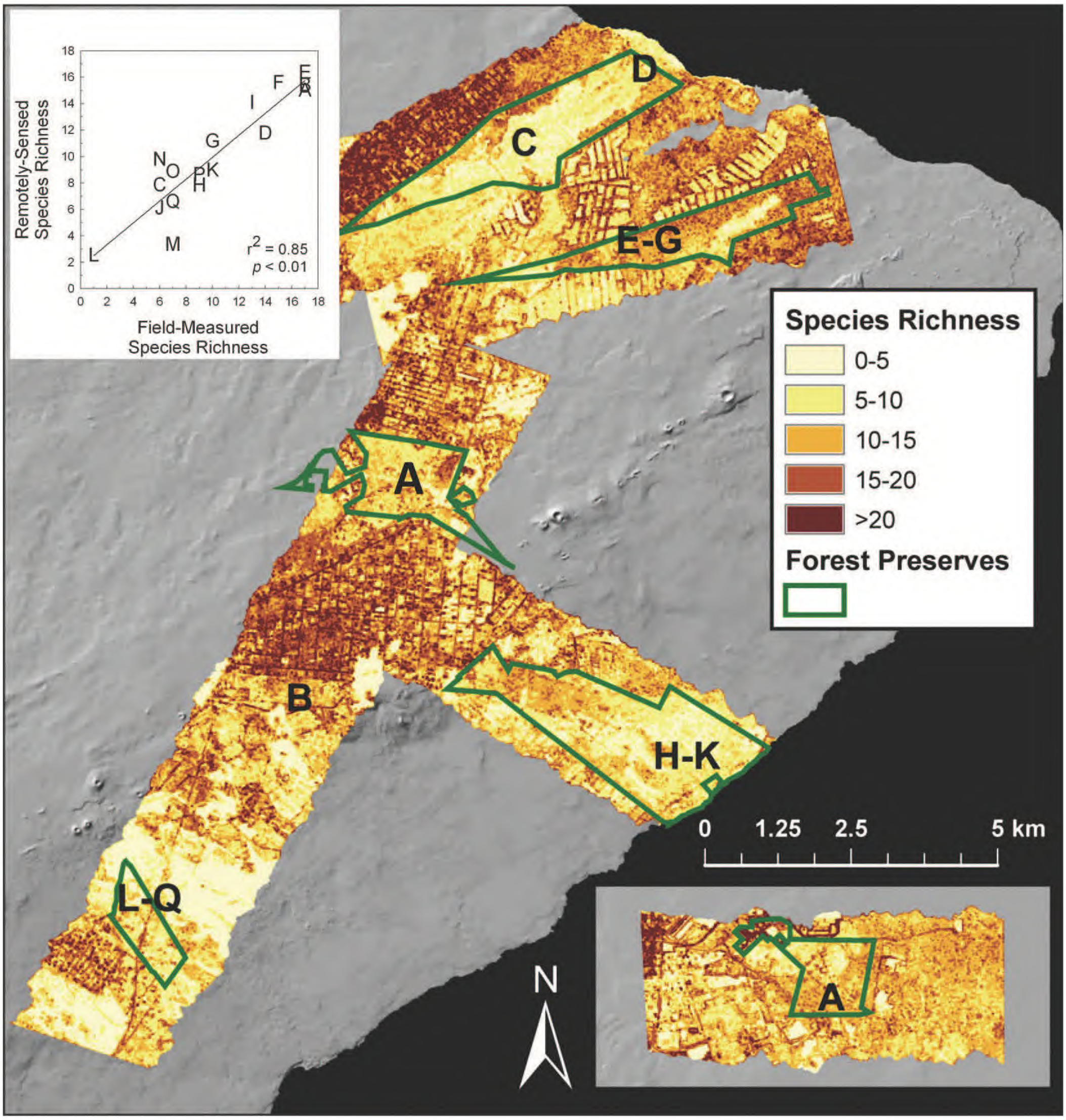


Figure 4. Maps of species richness (number of species per unit area) in lowland forests of the Puna District, Island of Hawai'i. In this set of examples, protected areas (green polygons) harbor relatively low-diversity native Hawaiian plant communities. Zones of higher species richness are found in communal areas harboring many exotic species. A–Q. Field validation sites as shown in the graph inset to the upper left. These maps were created using data from Carlson et al. (2007).

and compounds listed in Table 1. In general, spectral variance increases with chemical variance among co-existing organisms until an asymptote is reached (Asner & Martin, 2009). The saturation point at which adding organisms no longer leads to enhanced spectral or chemical variance is dependent upon the composition of an ecosystem, with climate and soils playing an important mediating role (Asner et al., 2009c; Féret & Asner, 2012b).

While species richness provides some insight into biological diversity, the holy grail of remote sensing is the ability to detect and map particular species (Innes & Koch, 1998; Nagendra, 2001; Turner et al., 2003; Saatchi et al., 2008). Aerial photographs have been used for years to estimate the cover and abundance of conspicuous temperate or plantation species (e.g., Swetnam et al., 1999), and some of that capability has been transferred to high-resolution spaceborne cameras (Thenkabail et al., 2004). Yet, standard imaging cameras have yielded little information on species distributions in tropical canopies. Detection of individual species depends almost entirely upon

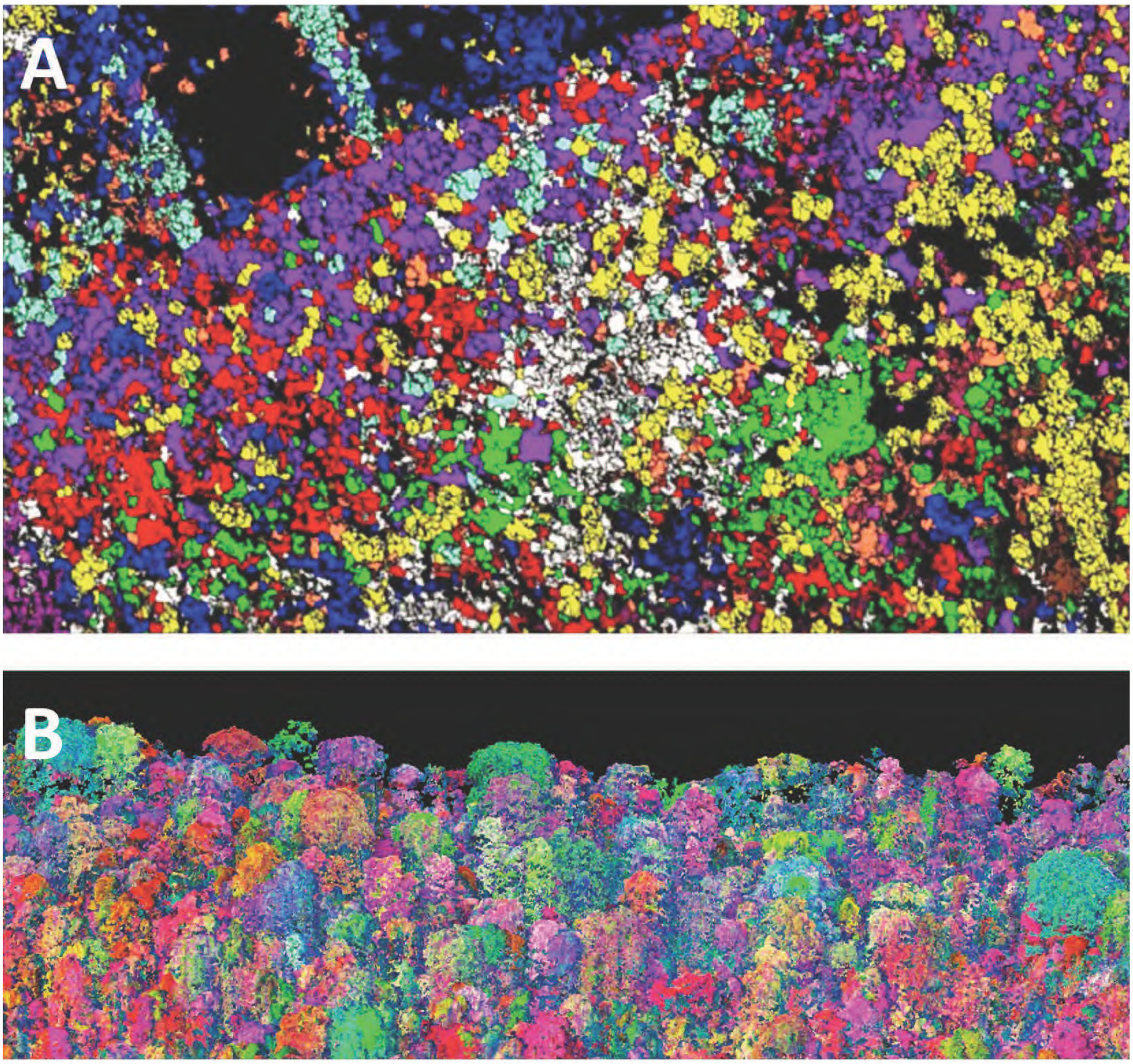


Figure 5. —A. Species classification in lowland Hawaiian tropical forest using airborne imaging spectroscopy. Black indicates non-forest areas. Each color represents a different species (Féret & Asner, 2011). —B. Structural, functional, and biological diversity in three dimensions as imaged by the Carnegie Airborne Observatory (CAO; <http://cao.ciw.edu>). This oblique view shows an Amazon rainforest canopy with colors indicating relative differences in the concentrations of growth, longevity, and defense chemicals among co-exiting canopies.

the degree to which the remotely sensed traits of an organism are unique to a given species on the landscape or within the ecosystem. Examples producing local uniqueness might include: (1) a nitrogen-fixing species (high foliar nitrogen, low LMA) against a community of non-fixers (lower nitrogen, higher LMA); (2) a bamboo species (tall, narrow open crowns, low LMA) against a community of tree canopies (wide, interlocking crowns, higher LMA); (3) light-demanding species (low LMA, low phenols/lignin) against a community of shade-tolerant species (high LMA, phenols or lignin), and so on. One of the keys to predicting which, if any, species might be unique on a landscape rests in understanding the traits among co-existing organisms. If the traits differ enough, then the species will be discernible; however, today we have a poor understanding of how many and under which conditions species might be discriminated.

What we do know comes from very recent studies at leaf and canopy scales. Asner and Martin (2009) first proposed that leaf spectra, once converted appropriately to chemical data, could be used to assess tropical forest compositional differences. This approach was extended to the canopy scale, both in modeling (Asner & Martin, 2008) and applied (Asner et al., 2008b, 2008c) work. Through this effort, it became clear that there almost always exists a set of species that can be classified from "organismic-resolution" spectrometer imagery such as is available

from the Carnegie Airborne Observatory (CAO; <http://cao.ciw.edu>). More recently, Féret and Asner (2012a) developed species classifications of humid tropical forest canopies using the spectral information measured by the CAO (Fig. 5A). This work has been nicely complemented by similar work in Costa Rican rainforests (Clark et al., 2005) and in several studies dealing with sub-tropical and temperate systems (e.g., Townsend & Foster, 2002; Bunting & Lucas, 2006; Lucas & Carter, 2008).

A case in point of species detection in tropical forests lies in the body of work focused on invasive rainforest species in the Hawaiian Islands. Detection and mapping of invasive species are key components of conservation and management efforts in Hawai'i, but the spectral and chemical separability of native, introduced, and invasive species had not been established until recently. Using organismic scale airborne imaging spectroscopy, Asner et al. (2008b) first identified the canopy spectral reflectance properties of seven common native and 24 introduced tree species, the latter group containing 12 highly invasive species. Spectral signatures of Hawaiian native trees were found to be almost universally unique from those of invasive trees and highlighted fundamental differences in the underlying canopy chemistry between the two groups. After the spectral and chemical signatures of native and invasive tree species were identified, Asner et al. (2008c) used the CAO to search for these species throughout a series of lowland to montane Hawaiian rainforests. The CAO combines imaging spectrometers with LiDAR, yielding three-dimensional spectral and structural maps that can be readily interpreted (Fig. 5B). The combined LiDAR-spectroscopic analysis highlighted the location and fractional abundance of invasive tree species throughout the forests, with field validation indicating $< 7\%$ error in the detection of invasive species with a canopy area of at least 7 m^2. This effort suggested that spectral and chemical signatures can be determined for species, which subsequently can be used to map them in airborne (and maybe someday, space-based) imagery. As a result of the Hawaiian studies, the Spectranomics Project (<http://spectranomics.ciw.edu>) was founded to inventory and analyze spectral and chemical signatures of thousands of canopy species throughout the world's tropical forests. To date, the Spectranomics Project has collected, measured, and catalogued more than 12,000 tropical forest canopy trees and lianas and is providing the baseline data for converting spectral signatures from the CAO and other airborne systems to chemical and biological diversity information (Asner et al., 2009c, 2011c, 2012b; Doughty et al., 2011; Colgan et al., 2012; Somers et al., 2012).

Finally, functional diversity (defined here as the spatial variance in functional traits among co-existing organisms) has increasingly been the focus of attention in remote sensing (Ustin & Gamon, 2010; Ollinger, 2011). One goal has centered on understanding spatial and temporal patterns of functional variance, with the intention of interpreting patterns in the context of ecological feedbacks, biogeochemical cycles, and evolutionary processes (Aguiar et al., 1996; Estes & Loveland, 1999). In tropical forests, functional diversity mapping has been difficult to put into practice, but field-based results already suggest that functional diversity is linked to gradients in soil fertility and climate (Townsend et al., 2007, 2008; Wright et al., 2007; Fyllas et al., 2009; Malhado et al., 2009; Girardin et al., 2010). In lowland Amazonian forests, Asner and Martin (2011) and Asner et al. (2014) recently extended this understanding into the remote-sensing arena by showing that a shift in canopy chemical traits, including those related to growth (e.g., phosphorus) and defense (e.g., phenols), is directly expressed in the spectral reflectance properties of the canopies. Yet, despite the systematic shift in both spectral and chemical traits, diversity among these traits within a community on a common soil type remains foremost dominated by taxonomy. That is, taxonomic control over chemical and spectral traits persists at the community level despite any synoptic-scale change in fertility conditions. More work is needed here to address the relative importance of environmental and phylogenetic contributions to functional patterns in tropical forests as detected using organismic-resolution remote sensing. The pay off from additional study will be a vastly improved understanding of biodiversity patterns and their underlying ecological and evolutionary drivers.

Macroscale Tropical Botany and Plant Ecology?

When conducting field work in the tropics, it does not take long to recognize the extreme challenges in carrying out ground-based carbon inventory, finding new species, or understanding spatial and temporal changes in community composition. Field botanists and ecologists have led the way thus far, yet there is much more to do, and field work alone will likely not yield the data required to make the scientific breakthroughs or conservation advances needed at the macroscale. Yet, remote sensing will not replace the critically important role that field botany and ecology plays in identifying species and studying their distributions in tropical forests. Rather, remote

sensing should be seen as a technique that will eventually extend our view of tropical forest carbon stocks and floristic composition to scales that are intractable on the ground. How far will remote sensing go in this realm? The answer is based on at least three factors, each requiring much more research and development: (1) the correlation between phylogenetic diversity and plant chemical and structural traits; (2) the connections between plant traits and remotely sensed variables; and (3) the development and deployment of new remote-sensing technology to support plant trait mapping and diversity assessments. Continuing progress on all three fronts will provide a pathway forward as we seek the botanical and ecological patterns needed to improve our understanding of processes operating in tropical forests at various temporal and spatial scales. The necessary information is currently difficult to obtain, but it remains key to conservation planning, forest management, and resource policy development in this century of enormous environmental change.

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DEFORESTATION AND SUSTAINABLE MIXED-USE LANDSCAPES: A VIEW FROM THE EASTERN HIMALAYA[1]

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ABSTRACT

The interrelationships between smallholder agriculture, forest cover, and biodiversity loss have received insufficient research and analytic attention, though they stand at the center of the ongoing biodiversity crisis in tropical landscapes. Despite important advances in conservation science, knowledge generation remains fragmentary, and the formal institutions concerned with agriculture, forestry, social change, and climate change continue to work to a large extent in isolation, especially in developing countries. Drawing on our research program in the montane Eastern Himalaya of India, for example, two questions are explored: What types of landscapes can provide livelihood security for growing populations while maintaining healthy ecosystems? What kinds of knowledge, institutions, and policies will help us move toward land-use patterns that support livelihoods and protect biodiversity, given a regional economy based on small-scale agriculture? Here, we advocate for a greater integration of knowledge types (scientific, traditional, and participatory) and institutions (formal and informal, government and community) to foster better planning in order to reconcile several functions at the landscape level, including smallholder agriculture, the production of ecosystem goods and services, and the protection of biodiversity.

Key words: Biodiversity loss, ecosystem services, Eastern Himalaya, land-use patterns, smallholder agriculture, sustainable landscapes, usable knowledge.

THE PROBLEM: TROPICAL BIODIVERSITY LOSS

The last few decades have witnessed tremendous advances in conservation science and increased awareness of the importance of biodiversity and ecosystem services (defined by the Millennium Ecosystem Assessment [2005] as the "benefits people derive from natural ecosystems," and by The Economics of Ecosystems and Biodiversity [TEEB; 2010] as the "contributions of ecosystems to human wellbeing"). Policies and governance for biodiversity conservation are improving slowly and unevenly. However, there is little evidence that the rate of biodiversity loss in the form of deforestation or forest degradation, especially in the tropics and the subtropics, has declined. Hansen et al. (2008) estimated tropical forest loss at 73,000 km²/year between 1990 and 1997, with a reforestation rate of only 10,600 km²/year during the same period (the reforested areas including both naturally regenerating forests and tree plantations). A more recent analysis of global forest loss between 2000 and 2005 found a total loss of 95,000 km²/year of humid and dry tropical forest over the five years, starting with 18,699,000 km² of total forest biome area in 2000 and amounting to a 2.5% loss of that total (Hansen et al., 2010). In the latter analysis, "forest" was defined as 25% canopy cover with trees at least 15 m in height. Because of the slow rate of growth in forest vegetation, increases due to regeneration or tree plantations were not included.

However, deforestation is a very gross measure of biodiversity loss. Selective logging, too, exerts a series of effects that may degrade not only the logged area but also adjoining areas. In the humid tropics, where commercial stems may be widely separated, Asner et al. (2010) estimated the overall area affected by selective logging to be 20 times that of outright deforestation. Invasive species, too, are responsible for the degradation of forests in many tropical regions. Remote sensing methods currently used to monitor global forest loss are unable to detect the more subtle changes in forest condition due to these forms of forest degradation, though they can also result in biodiversity loss. (See Kimothi et al. [2010], however, for evidence of steps toward this technical capacity.)

Much of the net reduction in tropical forest cover until now has been due to shifting land-use patterns. In the future, climate change is likely to exert major additional and complicating impacts on tropical

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forests. Asner et al. (2010) calculated that by 2100, the combined effects of climate change and land-use change (e.g., agriculture and selective logging) could negatively affect at least three fourths of tropical humid forest: up to 81% in Amazonia, 74% in the Congo Basin, and 77% in Asia-Oceania. Thus, according to Asner et al.'s modeling exercise based on 16 general circulation models (GCMs), by the end of this century only around 20% of the tropical humid biome is likely to remain unaffected by combined climate and land-use changes.

CHANGING KEY DRIVERS

Agriculture clearly remains one of the key drivers of land-use change (Lambin et al., 2001). Growing human populations, increasing consumption, and rising competition in land uses for biofuels and livestock feed production will inevitably continue to exert pressure on tropical forests (Food and Agriculture of the United Nations [FAO], 2011). At the same time, smallholder agriculture has been a major driver of deforestation in the tropics, especially in Asia in the 1970s and 1980s (Bawa & Dayanandan, 1997). Yet, the contemporary magnitude of deforestation by small farmers in tropical Asia remains controversial and difficult to estimate. Smallholder activity in the tropics is fluid, especially in areas remote from population centers and close to forest margins. It responds to a shifting array of social, economic, and ecological cues, many of them remotely generated. Global and regional measures of forest loss, thus, tend to conceal the complexity and dynamism of forest change patterns.

THE CONTEXT: EASTERN HIMALAYA

We have chosen the Eastern Himalayan region in India to address the intersecting issues of deforestation, poverty, and agriculture within the context of conservation of biodiversity and associated ecosystem services. In the Darjeeling Hills of northern West Bengal, in the Indian state of Sikkim and in the Nepali border province of Ilam, lowland humid evergreen forests (dominated by *sal*) mount steeply through species-rich moist maple-oak-rhododendron forests to high-altitude rhododendron and alpine conifer associations. Strictly speaking, much of this area falls at the borderline of the tropics due to its altitude-dependent winter frosts and snows. The regional "hill culture" is dominated by Nepali, Tibetan, and Bhutanese influences, rather than by the Indian culture of the "plains." Nevertheless, both natural and traditional social/agricultural systems throughout the Eastern Himalaya have been regulated principally by the strong annual monsoon, and the flora is a varied mix of Indian Peninsular, Indo-Burmese, Palearctic, and Far Eastern elements. Here, biodiversity-rich montane landscapes are dominated by small farms, generally less than 0.5 ha each. These are interspersed with forests and other land uses, primarily commercial tea plantations. Over the last several decades, smallholder agriculture and the collection of fuel wood and fodder from the forest have been major drivers of regional land-use transitions. Behind these drivers lie population growth, trade, and inadequate governance.

The main argument here is that the interaction between small-scale agriculture and biodiversity change remains largely unexplored with reference to two questions: What types of landscapes can provide livelihood security for growing populations while sustaining critical ecosystem services? What types of knowledge, institutions, and policies may help us move toward land-use patterns that support livelihoods and protect biodiversity, given a regional economy based on small-scale agriculture?

We believe that research and action focused at the interface of biodiversity and small-scale agriculture have the potential to point the way toward effective biodiversity conservation measures in populous tropical nations. Although our analysis is site-specific, the conclusions we draw are applicable to other parts of the world with similar landscapes and can be applied to a variety of social and economic contexts.

People, Land Uses, and Landscapes

The total magnitude of deforestation in India in general, and in the Himalayas in particular, remains uncertain, due primarily to conflicting estimates. The Forest Survey of India (FSI), a government agency, makes an assessment of land cover change every two years. Based on these assessments, it appears that net Indian forest area has remained stable over recent years (FSI, 2009). However, the latest analysis of deforestation by independent researchers indicates an annual deforestation rate of 1.5%–2.7%, which is consistent with earlier independent estimates (Puyravaud et al., 2010).

For the Himalayas, data are scarce. The few available studies indicate that forest loss and degradation have continued until recently (Prabhakar et al., 2006; Pandit et al., 2007). Baland et al. (2010) found that over one half of a forest sampled in Himachal Pradesh and Uttaranchal was highly degraded in terms of reduced canopy cover and increased cutting of branches and twigs for fuel and fodder, although basal area remained relatively



Figure 1. Typical mixed-use agricultural landscape in Darjeeling Hills, northern West Bengal, 2011.

unaffected. Smaller-scale studies, however, show countervailing and sometimes contradictory trends. The analyses by Nagendra et al. (2009) for a protected area and forest reserve between 1990 and 2000 indicated an increase in forest area in some parts of the reserve but also increased fragmentation in the same areas. Our own analysis for a different region in the Himalayas indicates continuing deforestation from 1990 to 2005 (Chaudhary, Shrestha & Bawa, unpublished data).

Throughout the Eastern Himalayas, population growth has been pushing farm boundaries into forests. As always, grazing and gathering of biomass for fuel and fodder advance ahead of the agricultural "frontier" itself. In recent years, at the landscape level, hydroelectric dams and associated infrastructure such as roads and tourism developments have become major additional drivers of biodiversity change. In many areas, the forest understory is dominated by invasive native and alien species of bamboos and shrubs. These also invade landslips and logged forests.

Sustainable Landscapes

Within this context, what specific meaning might the concept of a "sustainable landscape" carry? The term has entered common usage, but like the concept of sustainability itself, it is poorly defined due to multiple competing definitions. We define sustainable landscapes as those covering tens of square kilometers or more that sustain food production, provide biodiversity-rich habitats, and maintain ecosystem services (fresh water, soil retention and fertility, fuel wood, fodder, and other non-timber forest products [NTFP]) for current and future generations. Other attributes based on biophysical characteristics include provision of livelihood security and resilience to climate change. A further category of attributes, including social justice and equity of resource access, belongs more particularly to the institutions that govern sustainable landscapes, yet these, too, will presumably be expressed in the spatial organization of sustainable land uses (Ostrom & Nagendra, 2006; Turner et al., 2007).

Figure 1 shows a typical landscape of the Singalila Range of the Eastern Himalayas in 2011. The landscape represents a mix of agricultural production with fairly high biodiversity value and the continued maintenance of important ecosystem services. A hundred years ago this landscape consisted almost exclusively of forest. Today, many small forest fragments, orchards, and individual trees are interspersed among houses, small agricultural fields, gardens and terraces, patches of degraded vegetation,

etc. Forest copses generally remain on the steepest slopes, especially along the streams that have cut steep-sided channels through the erodible sedimentary soils. One of the most noticeable aspects of these Himalayan landscapes is the extreme steepness of much of the topography. It forces human habitation to cling perilously, and ingeniously, to the least precipitous slopes available.

Socio-ecological interactions and political and economic forces have generated the current configuration of land uses over several generations. Although this landscape appears to be "balancing" several functions (i.e., "reconciling" rather than "optimizing"), it is a landscape in flux and under considerable demographic and extractive pressure, supported by fragile regulatory frameworks. Whether or not further deforestation can be curtailed in this landscape will largely depend upon efforts to alleviate poverty, biophysical factors, future population growth, flows of goods and services to and from surrounding landscapes, socio-ecological resilience to climate change, and other potential challenges, and on the enabling knowledge, institutions, and policies.

POVERTY ALLEVIATION

The average annual income of rural households such as those shown in Figure 1 is approximately Rs48,000 (under US$800 in 2014), very close to the poverty line as defined by the Government of India. Landscapes such as these cannot be considered sustainable when the vast majority of their inhabitants live in poverty. The spatial correlation between biodiversity and poverty is widespread and not confined to the Eastern Himalayas (Sachs et al., 2009). With limited land, the few options to raise household income include increasing the productivity of the land and producing a diversified portfolio of goods and services (Bawa et al., 2012). However, the effects of poverty on forests are complex and variable (Chomitz, 2007; Roe, 2008), and poverty alleviation cannot independently make the landscape sustainable.

BIOPHYSICAL FACTORS

The long-term productivity and ecological efficiency of any landscape-scale arrangement will depend partly on how closely the distribution of land uses corresponds to the underlying productive potential of particular patches, including soil nutrient availability, hydrology, prevailing local climate factors, slope, and aspect, etc. Natural forest in the Eastern Himalaya is highly diverse, patchy, and dynamic, due both to the variety of volcanic and alluvial soil types and to frequent landslips, which expose or truncate soils on steep slopes with thin organic soil horizons (Singh & Singh, 1981). The region experiences heavy monsoon-season rainfall, and frequent seismic activity also contributes to soil erosion (Burbank et al., 2012). Agriculture has extended onto high and steep slopes requiring the adoption of labor-intensive measures such as terracing and earth bunds to prevent surface soil erosion (Mandal & Sharda, 2011). While short-term productive potential is probably not being maximized in our landscape, medium-productivity rates together with a degree of longer-term security may have been attained. Questions about biophysical limits to sustainability, however, require empirical data.

POPULATION TRAJECTORY

During the last 50 years the population in the Eastern Himalayan states of India has tripled, though with a declining growth rate since the 1960s (Goel, 2005). In the rural landscape shown in Figure 1, our results indicate that population size has almost doubled during the same time period, primarily due to growth and multiplication of original households in the area rather than immigration. In Darjeeling District as a whole, the decadal population growth rate decreased from 24% in 1991–2001 to 14% in 2001–2011. In the adjoining state of Sikkim, the average growth rate from 2001 to 2011 was only about one third of the growth rate over the previous decade. In the Darjeeling Hills, for which detailed data are available only until 2001, population growth was unevenly distributed. Darjeeling town grew at 45% over the decade to 2001, and rural parts of the southern plains and the fertile agricultural areas around Kalimpong also grew rapidly (~26%), while there was slight negative growth (~1%) in the high hills toward Singalila, north and west of Darjeeling town (the site shown in Fig. 1). If the population stabilizes during the next decade or two, with a modest increase in the productivity of agriculture, such landscapes combining a mixture of agricultural lands and native forests and watersheds could be maintained.

FLOWS OF GOODS AND SERVICES

The rural landscape shown in Figure 1 is by no means a closed system. Along with the flow of human inhabitants, a variety of material stocks and ecosystem services flow both into and out of the region. The high slopes are still clothed in forest, from which dry-season water flows remain significant. Streams carry

sediments downstream that constantly nurtures and renews soils in river valleys and lowlands. Other significant services include pollinators and natural predators of pests. The aesthetic, spiritual, and religious importance of the Himalayan landscapes is enormous.

Ecosystem "services" are not invariably beneficial to human populations. While monsoon-season floods spread fertile sediments on agricultural fields throughout the Eastern Himalayan foothills, they can also cause considerable devastation. Wildfires clear forest understory and help improve the fertility of soils for planting. Yet protecting themselves from the destructive impacts of these natural forces is one of the primary goals of cultures worldwide. In the unprotected river valleys of the Himalayan foothills, the areas closest to the riverbanks are occupied by the most marginalized segments of society (e.g., Kalita et al., 2010). Risk of disastrous flooding is an ever-present aspect of their lives, and they are often a constituency for multi-purpose dams and other water development projects that promise some degree of human control over their environment. Similarly, biodiversity itself is no unambiguous "good" for those living in close contact with it. Many animals are destructive of crops or livestock. Others compete directly with people for high-value natural resources. Non-commercial plants may compete successfully with economically useful ones. People everywhere seek to increase their control over potentially dangerous natural forces.

Furthermore, protecting biodiversity and protecting ecosystem services are by no means synonymous. High levels of ecosystem services can often be provided through careful management of agricultural land, but managing agricultural landscapes for the associated ecosystem services does not automatically protect wild biodiversity (Buck et al., 2006). Thus, most people aspire to live in areas with controlled, predictable flows of useful natural services; this tends to mean multifunctional landscapes with mosaics of different land use intensities at the local scale.

There is a net flow of positive ecosystem services out of the Eastern Himalayan montane region (including water, soil nutrients, carbon sequestration, pollinators, and eco-tourism) as well as a variety of goods that directly support the agricultural system (Bawa et al., 2007). Local people contribute to the maintenance of these services while at times suffering from the disservices (in particular, floods and human-wildlife conflict). To the extent that they contribute to the maintenance of valuable services, individuals and communities may be said to be "producing" ecosystem services or habitats. A more formalized system of compensation by society, such as that is already in existence across the developed world in the form of conservation easements, subsidies, and offsets, would go a long way in maintaining these types of mixed-use landscapes (Naidoo et al., 2006; Wunder, 2007). This, however, puts a premium on institutions capable of organizing, assessing, and dispensing compensation, outputs related to the institutional frameworks discussed in a later section.

CLIMATE CHANGE AND RESILIENCE

To a large extent, resilience to climate change remains an undefined parameter (see, e.g., Moench and Dixit [2007] for a summary of the issues). Making the concept of sustainable landscapes operational will, thus, require the development of new kinds of knowledge and institutional forms. In the Eastern Himalayas, residents are aware of changing meteorological trends, and they are interpreting them as the onset of climate change (Chaudhary & Bawa, 2011; Chaudhary et al., 2011). Inhabitants are beginning to adapt their agricultural strategies to the changing conditions, generally making adjustments at the household level and within the limits of the traditional response patterns and technologies available to them. If climate-related changes continue and exceed the bounds of historical variability, they will certainly place new stresses on institutional resources. In particular, the ability of government and non-governmental and community agencies to successfully communicate and cooperate when responding to extreme events will be challenged. In much of this region, extremes are expected to come in the form of increased flooding and landslides. However, there will likely be a good deal of local variability. Advances in the precision of regional level climate modeling are sorely needed.

Toward Sustainable Solutions: Knowledge and Institutions

In a thoughtful essay, Sayer et al. (2008) pointed out that conservation biology has become quite sophisticated in its ability to identify and quantify threats and to characterize the distribution and function of biodiversity, but it has placed much less emphasis on defining and articulating desired outcomes. Such outcomes require integration of knowledge across disciplines (biological, social, and political), knowledge systems (scientific and traditional), and spatial (regional and local) scales (Bawa et al., 2012). However, our ways of generating and distributing knowledge remain fragmentary. For example, even among the ecological sciences, land

use change, biodiversity change, and climate change are often studied as separate domains and are frequently monitored separately, especially in developing countries, though their drivers and effects are intimately intertwined. Livelihoods, agriculture, and biodiversity, too, remain conceptually separate, despite the fact that it is precisely the interactions among these domains (expressed through the actions of small farmers and policy makers) that generate the type of landscape shown in Figure 1.

Furthermore, the incentives to which small-holders respond are not necessarily amenable to observation and analysis by non-governmental organizations (NGOs) or government agencies not based in the region or with a long-term presence there. Assessing the potential of landscapes for sustainability requires information that can only be gathered through participatory processes. Consequently, our ability to generate usable knowledge to help civil society imagine, choose among, and move toward positive options has remained relatively limited.

The processes and institutional frameworks needed for producing integrated knowledge and action largely remain to be developed, particularly in developing countries (Bawa et al., 2008). Apart from generating relevant knowledge, we urgently need to develop institutions capable of making the links with action to support sustainable landscapes.

INSTITUTIONS FOR SUSTAINABLE LANDSCAPES

What is "usable knowledge"? By definition, it incorporates due consideration by society and particularly by policy makers (Clark et al., 2011). Because knowledge that contributes to sustainability is often still divided into separate domains, in many countries separate government agencies have to deal with agriculture, forestry, and water, to name a few. Furthermore, although markets, financial incentives, and disincentives play important roles in shaping landscapes, agencies dealing with natural resource management are rarely expected to concern themselves with the social factors underlying the patterns of natural resource use. When they are expected to integrate these factors, as in the administration of the Joint Forest Management Programs (India) and Community Forestry Programs (Nepal), for instance, they are not always well prepared to do so (Ghate, 2003; Lélé et al., 2003). Researchers, for their part, have focused productively on the ways in which formal institutions, such as legal tenure systems, land markets, and governance, contribute to landscape organization (Agrawal & Chhatre, 2006; Ostrom & Nagendra, 2006), but the roles of informal institutions, such as social and kinship networks, and the subject of customary rights have received less attention, perhaps because our ability to document their influence remains relatively undeveloped.

Globally, the formal institutions concerned with agriculture, forestry, biodiversity conservation, social change, and climate change tend to work in isolation. Moreover, in the tropics, such institutions still have little or no interaction with local people and local landscapes, even though the United Nations Millennium Development Goals, which incorporate targets of the Convention of Biological Diversity (CBD), call for just such integration. There is great scope in the developing world for knowledge-generating institutions to develop, nurture, and promote interdisciplinary knowledge (Bawa et al., 2008).

CONTRASTING MODELS OF LANDSCAPE ORGANIZATION

How are institutional frameworks reflected in specific patterns of landscape organization? Sayer et al. (2008) contrast a "European model" (consisting of mosaics of discrete land-use patches) with the less well-defined patterns typical of the tropics. Many landscapes in populous tropical areas, including those located around and even inside protected areas, are highly dynamic today (Nagendra et al., 2006). Land-use patterns respond rather sensitively to changing pressures from market forces and population movements. In the Tadoba-Andhari Tiger Reserve in central India, for instance, rates of deforestation and spontaneous reforestation nearly balanced out one another over a 12-year period (Nagendra et al., 2006), but during that time, as much as 16% of the forest area transitioned from one state to the other, either from forested to deforested, or the reverse. Thus, overall forest cover figures may conceal considerable dynamism in land-use patterns.

In contrast, the "European model" of landscape organization has, over several hundred years of relatively slow-paced economic and demographic development, moved toward increased stability of landscape uses at finer and finer scales (Bloch, 1966; Rackham, 1986; Blackbourn, 2006), the "European model" now affords individuals considerable decision-making powers over legally owned lands, but these rights are increasingly heavily qualified by government agencies (including supra-governmental EU agencies) assumed to represent the rights of non-local stakeholders to biodiversity and other ecosystem services, recreation, "heritage," or existence values, and so forth. Restrictions on resource use or development are often compensated by large-scale subsidy instruments, administered contractually (Wunder et al., 2008). This is also increasingly the North American model, particularly in areas such as

New England with a relatively long history of dense settlement. Thus, the dominant trend throughout the industrialized world over the last two generations has been toward greater degrees of state/civil-society planning in response to increases in population density and rising incomes (Schreiber, 1990).

Eastern Himalayan landscapes (particularly in India) are regulated by more general national restrictions on timber harvest, NTFP extraction, and commercial development. There is still relatively little capacity for cadastral services, but in principle, land development above 2000 m elevation is regulated by the Forest Department at the fine scale. In those cases, plots are mostly privately owned, though many areas are in dispute. Legal and illegal agricultural encroachment into forested areas continues to an unknown extent (Oli et al., 2013).

Throughout India, land tenure arrangements are in flux. The Forest Rights Act of 2006 may constitute a significant step toward settling the land tenure rights of a large number of socially marginalized forest dwellers, but it is still being implemented only gradually in many areas (Sarin & Springate-Baginski, 2010; Bawa et al., 2011). If the Act can be completely implemented, and if its prescriptions can in turn be strongly linked with community forest stewardship, employment opportunities, and strengthened collaborative governance initiatives, it will be a big step forward.

In order to fully realize this step, however, informal and community institutions will need to be strengthened almost everywhere. In particular, they will need to build their capacity for collaborative partnerships with government agencies at a larger scale, and the government agencies will need to extend their ability to work creatively with community groups rather than simply dominating them in "participatory" situations, as has too often been the case (Lélé et al., 2003; Nayak & Berkes, 2008).

Conclusion: Getting the Incentives Right

Biodiversity conservation in many densely populated tropical landscapes needs to be understood and pursued as an element, even a driver, of effective economic development. As we move forward, conservation and development can no longer be pursued on separate tracks (Boedhihartono et al., 2007). The importance of securing willing participation from individuals and communities cannot be overestimated if implementation of conservation policies is to be effective. Under conditions of ongoing change, the overall goal must be flexibility and adaptability in land-use patterns considered across whole landscapes; this will require much-improved institutional frameworks and skills in negotiation. Needs and wants (in the Eastern Himalayas and more widely) will only continue to grow in the coming decades. As long as there are natural resources to be extracted and transformed to meet specific desires of local and global economies, enterprising individuals will find ways to gather and market them. Conservation strategies that rely narrowly on protecting resources by restricting their use and consumption, thus limiting economic opportunity, are unlikely to succeed in the middle to longer terms. In order to achieve lasting success in biodiversity conservation goals, incentive systems are needed that can motivate people to protect and regenerate resources, including biodiversity, rather than merely consuming (or degrading) them. "Sustainable use" in rural areas will thus depend less on effective limits to *consumption*, than on effective incentives for the *production* of renewable resources. Any such system of incentives will need to motivate at least "some of the people all of the time" and "all of the people some of the time." Putting such incentives in place will demand a strong emphasis on building the capacity of institutions and key groups to participate actively in knowledge generation and in local land-use planning. It will require an explicit focus on a healthy rural economy in the broadest sense, going well beyond the present system of incentives focused narrowly on the dimension of financial returns.

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PAST ECOSYSTEM DYNAMICS IN FASHIONING VIEWS ON CONSERVING EXTANT NEW WORLD VEGETATION[1]

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Abstract

In the Cretaceous there were eight New World terrestrial ecosystems that were distinct or only shadowy versions of those present today. Now, 65 Ma later, 12 ecosystems constitute the recognizable subdivisions of the Earth's living and physical envelope. Knowledge of the intervening history is useful for conservation in several regards. It reveals how heterogeneous modern systems are in age and origin (e.g., lowland Neotropical rainforest formed between ca. 60 and 58 Ma; tundra and páramo between ca. 5 and 3 Ma), how individualized the response of component species to previous environmental change has been, and how levels of biodiversity have fluctuated through time. History provides approximate analogs for conditions anticipated in the near future and estimates of the biotic response. In addition, history documents that climatic events originally discovered and formerly associated with the high latitudes also affected tropical regions, and that discovery constitutes a valuable context for assessing previously held views about the stability of tropical communities and environments. There has been a rapid increase in the pace of both natural and human-induced environmental change and biotic response approaching modern times. Blurring the lines between neontology and paleontology, namely, conceptualizing ecosystems over Cretaceous and especially Late Cenozoic time, provides a dynamic view of these systems and furthers realistic strategies to conserve them.

Key words: Climate change, conservation, ecosystems, New World vegetation, paleobiology.

Ecosystems are the plants, animals, physical features, and climates that interact to define recognizable segments of the landscape. Modern ecosystems in the sense of angiosperm-dominated communities started to appear in the Early Cretaceous ca. 125 million years ago (Ma), and by the end of that period (ca. 65 Ma), they had diversified and radiated to become the principal component of the Earth's living envelope (Graham, 2010a; Friis et al., 2011).

Initially in the Cretaceous there were eight ecosystems recognized for the New World: the polar broad-leaved deciduous forest, notophyllous broad-leaved evergreen forest, paratropical rainforest, tropical forest, aquatic, freshwater herbaceous bog/marsh/swamp, mangrove, and beach/strand/dune. These developed into the 12 ecosystems recognized for the present interval of time: desert, shrubland/chaparral-woodland-savanna (caatingas and cerrado), grassland (pampas in Argentina), mangrove, beach/strand/dune, freshwater herbaceous bog/marsh/swamp (including the Pantanal of Brazil, Bolivia, and Paraguay), aquatic ecosystems, lowland Neotropical rainforest, lower to upper montane broad-leaved forest (mostly mid-altitude Latin America)/deciduous forest (mostly North America north of Mexico), coniferous forest, alpine tundra (páramo), and tundra. Some appeared early and were already present in the Cretaceous, such as aquatic with representatives of Nymphaeaceae, *Ceratophyllum* L., *Decodon* J. F. Gmel., *Trapa* L., *Pistia* L., and others (Graham, 2010a [appendix 2.1, 2.2], 2011, 2011 et seq.), while others came later with increased drying and greater seasonality beginning in the Middle Miocene (ca. 15 Ma) and formed shrublands, woodlands, savannas, grasslands, and the early versions of desert. Early versions of others, such as tundra and alpine tundra or páramo, appeared even later when cool temperatures combined with increasing altitudes in the Late Miocene and Pliocene beginning ca. 6–7 Ma and became established ca. 5–3 Ma. In some instances, the physical opportunity for the development of a particular ecosystem was present in the Cretaceous (such as coastal brackish-water habitats), but the appearance of modern mangrove communities had to await the migration of *Rhizophora* L. into the New World, which did not happen until the Middle Eocene (ca. 45 Ma; Germeraad et al., 1968); oak did not cross the Panama land bridge into northern South America until ca. 330,000 years ago to form the present-day oak-*Weinmannia* L. forest of the upper Andean forest belt (Hooghiemstra & van't Veer, 1999; van't Veer & Hooghiemstra, 2000).

Biodiversity and the importance of preserving it are being widely presented to the public (e.g., Clark & Poulsen, 2012); hence the inclusion of selective

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references from the popular media (e.g., Safina & Lewison, 2014). However, the reasons for the present high levels particularly of tropical biodiversity are complex (Kraft et al., 2011), and history reveals this level is not the result of a simple straight-line increase in the tempo of evolution and the accumulation of species through time. Estimating past levels of biodiversity is an inexact science, but Wing (1997) suggested that during the warm interval around the Paleocene/Eocene boundary ca. 55 Ma, widely separated areas of North America, like the northern Rocky Mountains and the Gulf Coastal Plain, experienced a short-lived decline in diversity. Wilf et al. (2003, 2005) believe that sometime around 52 Ma there was high diversity in warm parts of South America (Patagonia, Argentina). Hoorn (1994) reported 280 pollen types in Miocene river sediments from northwestern Amazonia, while Urrego-Giraldo (1994) recovered only half that number in comparable sediments of Holocene age from the same region; Wijninga (1996) detected a decline from the Miocene into the Quaternary in Colombia. Paleobiology reveals modern ecosystems are heterogeneous assemblages of disparate communities that appeared at different times (Cretaceous through the Mio-Pliocene), originated in different ways (in situ and near–in situ evolution vs. components arriving via migration), and experienced its own complex ebb-and-flow pattern of diversity and extinction (Colles et al., 2009; Davies et al., 2011; Lorenzen et al., 2011; Uyeda et al., 2011). A summary of New World ecosystem history is given in Graham (2011; see also Graham, 1999, 2010a, 2010b, 2012; Raven et al., 2011, and papers therein). In addition to past effects, there are the numerous and widely discussed modern impacts on ecosystems (Dizro et al., 2014; Gillis, 2014; Gillis & Chang, 2014; Pimm et al., 2014; Stokstad, 2014; Vignieri, 2014) due to human activities, such as farming, lumbering, grazing, burning, and construction (Allan, 2011; Barrett et al., 2011; Doxford & Freckleton, 2011; Hoffmann & Sgrò, 2011; Kodra et al., 2011; Lee et al., 2011; Laurance, 2012a, 2012b; Laurance & Balmford, 2013). Recent studies suggest that species are shifting to higher elevations at a rate of 11.0 m/decade and to higher latitudes at 16.9 m/decade in response to global warming caused in part by increasing atmospheric CO_2 concentration (Chen et al., 2011), and that this is resulting in an Amazon basin transitioning to a disturbance-dominated regime (Davidson et al., 2012). Ecosystems will increasingly fluctuate in the future through a combination of natural factors and anthropogenic changes in the environment (SANparks, 2010) influencing the distributions, ecological tolerances, and levels and patterns of biodiversity established in the past.

Past environments, lineages, and communities can serve as useful analogs for conditions anticipated in the future and for estimations of the biotic response. Atmospheric CO_2 concentration was ca. 300 parts per million by volume (ppmv) just before the industrial revolution in the early 1800s. Currently, that concentration has grown to 400 ppmv; it is expected to increase to 550 ppmv by 2050 and reach 1000 ppmv by the end of this century. Near the end of the Cretaceous, CO_2 concentration was ca. 1500 with its equable temperatures and mesic vegetation; it was 2000 ppmv at the Paleocene-Eocene Thermal Maximum (PETM) around 55.9 Ma when tropical tapir, crocodile, and banana-like lineages extended to within the Arctic Circle. The rate of carbon emission is estimated at 300–1700 million metric tons per year (Cui et al., 2011). Current emissions are 10 times that of the PETM. Atmospheric CO_2 concentration was less than 200 ppmv in the Middle to Late Pliocene when glaciers began covering much of the Earth (Zachos et al., 2008; Breecker et al., 2009; Royer, 2010). These figures (ca. 200–1700 ppmv) bracket values known during the past ca. 65 Ma between the Late Cretaceous and the Late Pliocene. They are also those expected in the near future depending on the balance between continued CO_2 emissions and cold glacial periods of future Milankovitch cycles. For conservation purposes, it is useful to know and effectively convey to the public that these fluctuations in the concentration of greenhouse gases, along with other factors, are clearly documented in the past and that they can and have drastically altered the climates and ecosystems of the Earth: "Climatic fluctuations and vegetation responses are particularly rapid at glacial-interglacial boundaries. There is typically a gradual buildup to glacial conditions, then a precipitous and chaotic drop into interglacial climates, a process Alley (2000) likens to switches and dials. For instance, after the Last Glacial Maximum (LGM) at ca. 18,500 years BP, Greenland rapidly warmed by ca. 15°C in a decade or less at ca. 11,500 years BP, and Leyden (1984) has documented this interval of climatic instability for Guatemala" (Graham, 2010a: 267). Mean annual temperature (MAT) in the Amazon basin dropped by 5°C–6°C at the LGM ca. 21,000 years ago and was associated with extensive drying (see later section). To get a feel for what a change of 15°C, or even 5°C–6°C, means, recall press coverage in the 1980s of Midwestern farmers carrying truck-loads of hay to their drought-stricken farmers in the southeastern United States.

The rise in temperature associated with that event was 0.5°C. Relicts of former climate change are being studied to reveal how plants may persist considering the major environmental changes anticipated for the future (Hampe & Jump, 2011; Zimmer, 2011).

History is further relevant to the still-debated issue of lowland Neotropical rainforest stability (Hooghiemstra & van der Hammen, 1998). Through the 1960s, the biogeographic literature was replete with opinions suggesting that where rainforest is climax today, similar vegetation had existed uninterruptedly since a very remote geologic period; the complexity of the rainforest could be explained in terms of the seasonal and geologic stability of the climate; and in views espousing the ancient and stable environments of the tropics (Richards, 1964; Ashton, 1969). After four decades of studying the modern biota it was concluded that this presumed stability might not be true (e.g., Vuilleumier, 1971), and the fossil record has confirmed it is not. For example, at the northern limits of the modern tropical rainforest in southeastern Veracruz, Mexico, where rainforest is climax today, a Pliocene flora from a lowland area shows it was not present in the relatively recent geologic past (Graham, 1976a, 1976b). As noted previously, independent evidence from oxygen isotope and other climatological studies indicate that the MAT at the LGM was ca. 6°C lower (Webb et al., 1997) and that it was considerably drier (Maslin & Burns, 2000; Filho et al., 2002). A new study covering the last 45,000 years BP from the lowland interior of tropical South America at Laguna La Gailba near the border between Bolivia (Province Ángel Sandoval) and Brazil (Mato Grosso), hydrologically linked to the Pantanal, also reveals drying at the LGM and, by implication, at multiple times during the Quaternary: "We provide unequivocal evidence that the climate at LLG [Laguna La Gailba] was markedly drier during the last glacial period (45.0–12.2 kyr BP) than during the Holocene. . ." and this "strengthens the body of evidence for widespread early mid-Holocene drought across tropical South America" (Whitney et al., 2011: 177). Current droughts of even short duration have a noticeable effect on Amazonian greenness (Xu et al., 2011; Davidson et al., 2012), suggesting a prominent role for riverine dynamics in diversification (Ribas et al., 2011). It is difficult to imagine that in the 2.5 Ma of the Pleistocene, with its 18 to 20 cold/warm and moist/dry cycles, rainforest remained more or less continuously present across the Amazon basin as has been suggested (for the relevant literature see Graham, 2010a: 500–511). The historical record documents that the rainforest ecosystem is an ephemeral, delicately balanced community that has undergone significant changes in range and composition. The controversial refugium theory suggesting stability appears resolved, or at least quiescent, but its potential relevancy to conservation is clear. Too great an emphasis on stability provides a basis for the rationalization that the rainforest can be impacted with impunity and will withstand or recover because it has always done so in the past. The telling point from history is that it was not stable over long periods of time, and once it is gone, it takes intervals measured in multiple human generations and geologic time to recover.

Recent geologic history documents and provides further insight into the fluid nature of habitats, environments, and biotas. Davis (1981, 1984) long ago introduced the concept of the individual nature of species migration in response to environmental change and the availability and different distribution potentials of the propagules, the coming and going of barriers, epidemic disease, and other factors (see also Stewart et al., 2010). The consequence of this observation is that, within limits, communities of individuals, i.e., ecosystems, experience changes in composition with each environmental reshuffling. Oak-chestnut forests in the central Appalachians have included chestnut for only 2000 years. Deciduous forests in Ohio were penetrated first by hickory and then by beech 4000 years later, whereas in Connecticut, beech arrived first followed 3000 years later by hickory. Beech and hemlock entered northern hardwood forests in the Appalachians at about the same time, but hemlock arrived 2000 years before beech in New Hampshire and 2500 years before beech in upper Michigan (Davis, 1981, 1984). Beginning most recently at ca. 12,000 years ago and continuing off and on during the Holocene, there was a unique association of black ash and spruce in the upper Midwest that is no longer present today. As noted previously, an example from the tropical latitudes is the characteristic Upper Andean oak-*Weinmannia* forest that has contained oak for only the last 330,000 years, the last one eighth of the Pleistocene, because oak, although present to the north for millions of years, was able to cross into South America only after completion of the Panama land bridge, appearance of the moderate highlands, and the cooler temperatures of the Late Tertiary and Quaternary.

A recent study from Peru along the margin of the Quelcaya ice cap gives a modern patina to how changeable communities can be over relatively short periods of time with each climatic reshuffling, especially in regions with significant topographic relief. Gould et al. (2010) report that layers of

vegetation about 4500 years old have been progressively uncovered since 2002 with retreat of the ice. The rate of melting is up to 60 m/yr in some places. Using the chloroplast *trnL* intron, they found fossils of at least five plants in the now dry and cold habitat at 5200 m elevation whose modern counterparts presently occur in wetlands around 4000 m. These findings suggest a community moving up-slope during earlier warm intervals, covered by ice during a cold period with components moving down-slope, the fossil remains exposed during the present warm period, and components then moving up-slope to their former habitat—all happening within the past 4500 years. The study also provides a new technique for gauging the quickening pace of change in climates and biomes (for other studies on the pace of environmental change and the biotic response, see Hampe & Jump, 2011; Clark et al., 2012; Fraser, 2012; Tingley & Huybers, 2013).

There are other implications of geohistorical data for conservation efforts and how the topic should be presented to the public. One implication is that Quaternary climatic events discovered in the high northern latitudes, and later demonstrated for the high southern latitudes, also occurred and affected the biotas of tropical regions. The Younger Dryas and Heinrich events are now being reported from Brazil and other places (see Graham, 2010a: 409–446). Another implication is that earlier concepts about the long-term, leisurely pace of these changes on scales of hundreds of thousands of years are inaccurate. In older textbooks on glacial geology, four cold periods are described alternating with three warm periods, each lasting ca. 175,000 years, for a Quaternary interval of ca. 1.2 Ma. The Quaternary is now extended back to ca. 2.5 Ma with 18 to 20 cold periods occurring on the Milankovitch eccentricity cycle of 100,000 years with other cycles of ca. 41,000 and 19,000 years. Variations in ocean circulation impose additional shorter-term cycles called Heinrich events of a few to several thousand years culminating in cold intervals, and the cool to cold Dansgaard–Oeschger events of a few thousand to several hundred years. The Younger Dryas cold event occurred between ca. 11,000 and 12,000 years ago, the mid-Holocene warm interval 6000–8000 years ago, and the Little Ice Age 1300–850 years ago, which was itself interrupted by the Medieval Warm Period 1200–800 years ago (Fagan, 2004). Many of these fluctuations are correlated with events in human history (Fagan, 1999, 2000, 2004; Alley, 2000; Flannery, 2001, 2005; Beerling, 2007). It was in the Medieval Warm Period, for example, that the Vikings were able to make their voyages of discovery to North America. In the Little Ice Age, they were forced to abandoned the settlements; Washington suffered his near-fatal winter at Valley Forge; and the citizens of London were able to walk across the solidly frozen Thames River supporting tents, shops, cafes, and small buildings. The eruption of Mount Tambora in Indonesia in 1815 (Wood, 2014) produced the following "year without a summer," during the winter of which Mary Shelley, confined to quarters near Geneva with her husband the poet Percy Shelley, Lord Byron, and others, wrote for diversion the novel *Frankenstein*. In the book, as opposed to the movie version, the prevailing cold and ice of the summer-less year play a prominent role in both the opening and closing chapters. In essence, relatively recent climate changes are being revealed as far more rapid, intense, and widespread, even in the tropics, than suspected only a few years ago.

So what is the importance of these data on near-time and deep-time history for conservation policy (Blackman et al., 2014) and the preservation of ecosystems? The fact that they are important is evident from the extensive recent literature (e.g., Balmford, 1996; Swetnam et al., 1999; Burnham, 2001; Bruzgul et al., 2005; National Research Council, 2005; Jackson & Erwin, 2006; Willis & Birks, 2006; Froyd & Willis, 2008; van Leeuwen et al., 2008; Carrasco et al., 2009; Dietl & Flessa, 2009; Keane et al., 2009; Kowalewski, 2009; Gibbons, 2010; Willis et al., 2010; Barrett et al., 2011; Robinson, 2011; Valdes, 2011; Zeebe, 2011; Gillson & Merchant, 2014). Dietl and Flessa (2011: 30; Jaramillo & Cárdenas, 2013) stress that "inclusion of geohistorical data in our decision-making process provides a more scientifically robust basis for conservation policies than those dependent on short-term observations alone." It is easily possible to convey to the public—indeed, it has been conveyed to the public—that conservation involves primarily setting aside and prohibiting access to parcels of land housing a rare, threatened (Banerjee, 2013; Cashion, 2013; Gewin, 2013; Gibson et al., 2013; Josse et al., 2013; Säterberg et al., 2013), or otherwise important species, or to a particular ecosystem, with the goal of preserving it like it is in perpetuity. Examples include stands of *Podocarpus* L'Hér. ex Pers., *Araucaria* Juss., *Nothofagus* Blume, or other vegetation types in national forests; the shrublands of *Acaena* Mutis ex L., *Polylepis* Ruiz & Pav., and *Puya* Molina and *Calamogrostis* Moench grasslands in protected areas on the páramos; and coastal regions (Harriman, 2013; Liu et al., 2013; Nature, 2013a, 2013b). If this is the impression being given, a flaw in that strategy can be claimed by those opposed to conservation efforts by citing numerous references showing that species,

habitats, and ecosystems have come and gone over time. The conservation of hotspots (Joppa et al., 2011) interconnected by corridors (Robbins, 2011), monitored by remote sensing (Lucero, 2013), and inventoried through adequate and continually updated collections (Feeley & Silman, 2011) is immensely important for allowing time to collect, study (Ladle & Whittaker, 2011; Newbold, 2011), and preserve existing representatives, propagules, and DNA (Friedman, 2013; Griffith et al., 2013; Jenkins et al., 2013). Equally important are restoration efforts (Seddon et al., 2014) aimed at repairing the damage, assessing the impact of indigenous people (Luzar et al., 2012; Luzar & Fragoso, 2012; Gorenflo et al., 2012; Nature [editorial], 2012; Iwamkura et al., 2014), and, in the process, generating a better understanding of the composition, interaction, and functioning of species (Caro, 2013; Simberloff & Genovesi, 2013; Thomas, 2013) and ecosystems (Newbold, 2011; Newbold et al., 2012; Keenan et al., 2013). Additionally, however, when the dynamics of history, for example in the Anthropocene (Ruddiman, 2013), are incorporated into concepts about extant communities and publicized, a more complete, accurate, and defensible perspective of conservation and the nature of climate change (Blair et al., 2012; Clark et al., 2012; Gillis, 2013a, 2013b; Greenwald et al., 2013; Mace, 2013; Nature, 2013c, 2013d; McNutt, 2013; Tingley et al., 2013; Garcia et al., 2014) can be presented to the public; namely, that what we are actually or additionally doing is setting aside way stations. The biotas within these sites will be temporary because of both anthropogenic (Laurance, 2012a, 2012b; Laurance et al., 2012) and non-anthropogenic causes. From a geologic perspective, ecosystems of varying composition will be passing through them and at an ever-quickening pace. The essential point is that these way stations be available in the future to accommodate the new arrivals.

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EMERGING THREATS TO TROPICAL FORESTS[1,2]

William F. Laurance[3]

ABSTRACT

I highlight new and emerging threats to tropical forests, the world's most biologically diverse ecosystems. The drivers of tropical forest destruction and key perils to biodiversity have changed over the past decade and will continue to evolve in the future. Industrial drivers of forest conversion, such as logging, large-scale soy and cattle farming, oil-palm plantations, and oil and gas development, have escalated in importance, buoyed by rapid globalization, economic growth, and rising standards of living in developing nations. Biofuels are likely to grow rapidly as a driver of future forest destruction. Climate change is similarly emerging as a potentially serious driver of change in the tropics, and many species, including certain amphibians, primates, and plants, are being harmed by emerging pathogens. In general, old-growth forests are vanishing rapidly and being replaced by fragmented, secondary, and selectively logged forests, particularly in impoverished tropical countries. Road expansion continues apace and is increasingly imperiling the world's last tropical frontiers. Human population growth, especially in developing nations, remains an important underlying threat to forests. These various environmental dangers often operate in concert, magnifying their impacts and posing an even greater threat to tropical forests and their biodiversity.

Key words: Biodiversity, biofuels, China, climate change, deforestation, emerging pathogens, human population growth, industrialization, logging, overconsumption.

Tropical forests are important for many reasons—for sustaining biodiversity and indigenous cultures and for providing an array of valuable ecosystem services, such as storing carbon, limiting soil erosion, and reducing downstream flooding (Raven & Williams, 1995; Laurance, 1999; Bradshaw et al., 2007). Nearly one half of the world's tropical forests have been lost in the last few centuries and, at present, another 10 million hectares or so of native forest are being felled annually, the equivalent of roughly 40 football fields per minute (Laurance, 2010). Beyond this, many native forests are being modified, sometimes seriously, by threats such as habitat fragmentation, selective logging, overhunting, intense harvests of fuelwood and other natural products, and surface fires (Cochrane & Laurance, 2002; Peres et al., 2006; Laurance et al., 2011a).

The net effect of this constellation of changes is that many landscapes once dominated by old-growth forest are quickly becoming dominated by humans. In such landscapes, old-growth forests, if they persist at all, typically remain only in small, isolated fragments embedded within expanses of modified land, such as farms and human settlements, secondary or logged forests, and exotic-tree plantations. The future of tropical nature may well depend on the capacity of native species to persist in human-dominated landscapes and within protected areas that have become isolated and diminished as the lands surrounding them are altered by human encroachment (Laurance et al., 2012).

The proximate and ultimate (underlying) drivers of land-use change in the tropics are complex and continually evolving (Rudel et al., 2009). Understanding these drivers is important if one wishes to devise and implement effective conservation strategies. Here, I identify some emerging threats to tropical forests and their biota and briefly highlight some of their implications for nature conservation. The focus is on threats or trends that have increased markedly in the past decade or are looming just on the horizon.

INCREASING GLOBALIZATION AND INDUSTRIALIZATION

The ultimate drivers of deforestation are changing. In the 1980s and 1990s an expanding human populace, as manifested by hundreds of millions of small-scale farmers and rural residents living in tropical frontier regions, was often seen as the primary driver of forest loss (e.g., Myers, 1993). More recently, the situation has become more complicated. Industrial drivers of deforestation, such as large-scale agriculture, plantations, and ranching,

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[2] The author of this paper wishes to make it known to readers that the literature cited herein was up to date at the time that the manuscript was accepted for publication, in June 2013. Unfortunately, due to an extended interval between acceptance of the paper and its publication in 2015, this literature is no longer fully up to date. The author has updated two key references but not the entire paper.

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have risen sharply in importance (Butler & Laurance, 2008; Rudel et al., 2009). Other industrial activities, such as selective logging, infrastructure expansion, and oil, gas, and mineral projects, are promoting a proliferation of roads in frontier regions (Laurance & Balmford, 2013), which also promote forest loss (see below).

These trends are being driven by increasing economic globalization as well as by a rapid increase in industrialization in developing nations (Millennium Ecosystem Assessment [MEA], 2005). The rising importance of industrial drivers is provoking several important changes in the tropics. First, it appears to be accelerating the per-capita rate of forest loss in some regions (Wright & Muller-Landau, 2006). This is occurring because forests are increasingly being felled by bulldozers and other heavy equipment, rather than by small-scale landowners armed only with machetes and chainsaws. Second, it may tend to de-link the historically strong relationship between a country's population density and its remaining forest cover (Laurance, 2007a). A country like Gabon or Papua New Guinea, for instance, does not need to have a high population density to effect considerable deforestation when virtually every cut log the country produces is being rapidly exported to China (Laurance et al., 2006, 2011b).

Another important implication of increasingly industry-driven deforestation is that it affects conservation strategies. When deforestation was mostly being driven by small-scale farmers, this led to the emergence of strategies such as Integrated Conservation and Development Projects, which are designed to advance environmental conservation by promoting more sustainable rural livelihoods (e.g., Johannesen & Skonhoft, 2005). Today, however, the focus is shifting to some degree away from the myriad rural farmers to a far smaller number of large, resource-exploiting corporations (Butler & Laurance, 2008; Laurance, 2009a; Rudel et al., 2009). Many of these corporations have international stockholders and seek to export products to industrial nations where consumers tend to care about environmental sustainability. This raises the prospect for "bad corporate citizens" to be pressured by environmental activists to clean up their acts, or face the consequences of potential boycotts and a decline of their market share and corporate image.

Industrialization will surely continue to grow as scores of developing nations, such as China, India, Brazil, South Africa, and many others, continue to expand economically. The MEA projects a 300%–500% increase in global industrial activity by the year 2050 (MEA, 2005).

Roadway Expansion

We live in an era of unprecedented road and highway expansion. Many tropical regions that had been remote and largely inaccessible just one decade ago have now been penetrated by networks of roads (Laurance et al., 2009). For instance, major new highways are now criss-crossing the Amazon basin and South America (Killeen, 2007), and more than 50,000 km of new logging roads were recently detected in the Congo Basin (Laporte et al., 2007).

The problem with roads penetrating into tropical frontier regions is that their impacts often extend far beyond the road surface itself. Far too often, roads unleash a Pandora's Box of environmental problems that are difficult or impossible for governments to control. These include illegal land colonization, rampant land speculation, and influxes of illegal loggers, hunters, and miners (Laurance et al., 2009, 2014). As a result, fires and deforestation tend to increase dramatically near roads (Laurance et al., 2002; Kirby et al., 2006; Adeney et al., 2009). Such effects are especially severe and extensive around major paved highways, which provide year-round access to forest regions and tend to spawn large networks of secondary and tertiary roads and development pressure in general (Laurance et al., 2002; Kirby et al., 2006).

By dramatically increasing physical accessibility to forests, road expansion is one of the most important factors determining the magnitude and rate of forest destruction. For instance, road expansion is projected to have far-reaching effects on the future patterns of forest disruption in the Amazon (Fig. 1) (Laurance et al., 2001; Soares-Filho et al., 2006). It is also important to emphasize that road expansion is an activity that is highly amenable to policy modification (Laurance & Balmford, 2013). For instance, carbon-offset funds might be used to promote the rerouting or closure of frontier roads in vulnerable regions in order to reduce deforestation and atmospheric carbon emissions. A list of the world's most environmentally destructive tropical roads was provided in Laurance et al. (2009).

China's Role in Illegal Logging

In the tropics, one of the most profound changes in the last decade is the stunning rise of timber consumption by China, which has completely transformed tropical timber markets. China is now overwhelmingly the biggest global consumer of tropical timber, importing around 40 to 45 million m^3 of timber annually (Laurance, 2008a). In layman's terms, this means that more than one half of all

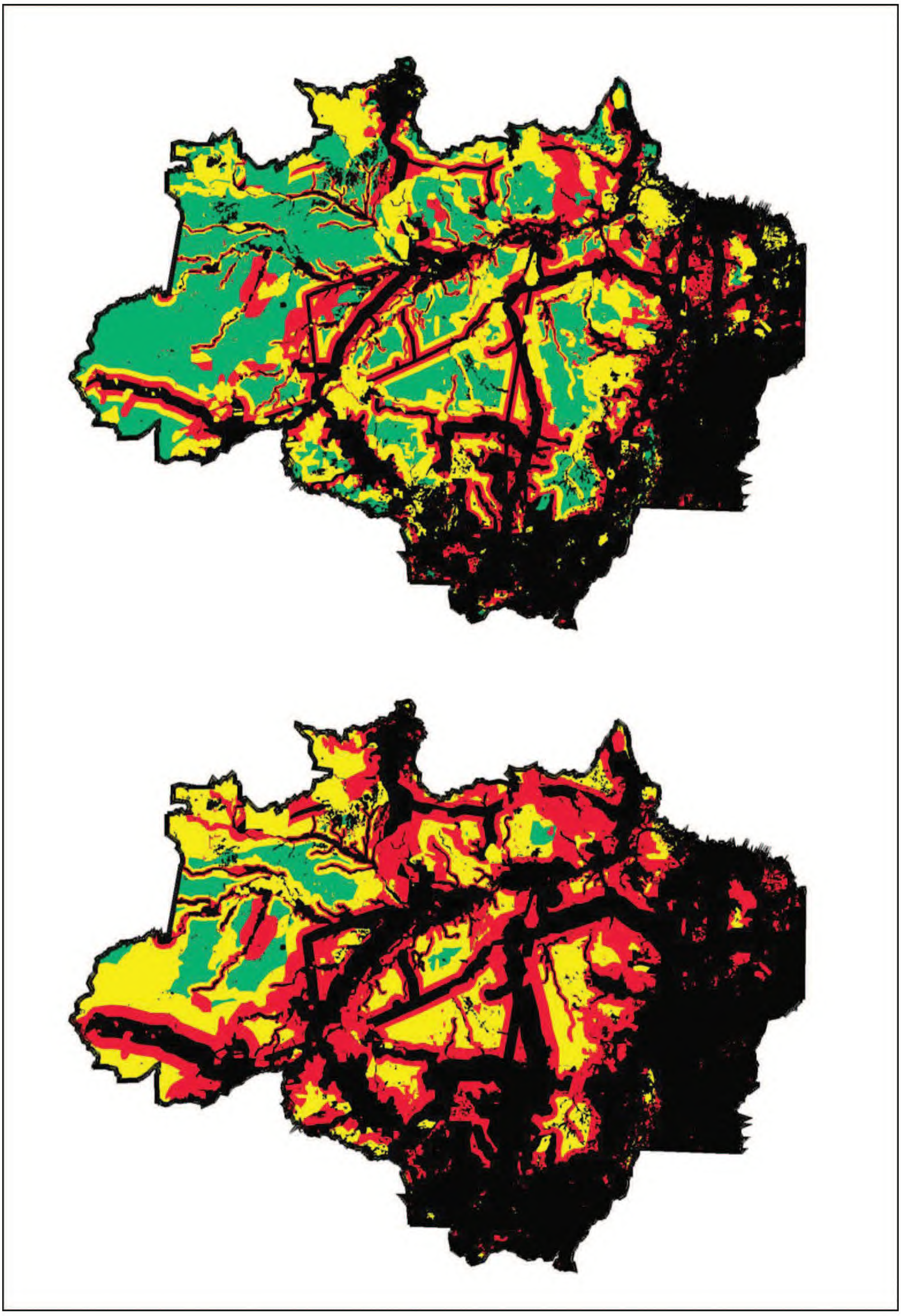


Figure 1. Roads are projected to have a major impact on future patterns of forest loss, fragmentation, and degradation in the Brazilian Amazon, which contains about 40% of the world's remaining tropical rainforest (~3.35 million km^2). Shown are "optimistic" (above) and "non-optimistic" (below) scenarios for the region in the year 2020 (black is deforested or heavily degraded, including savannas and other nonforested areas; red is moderately degraded; yellow is lightly degraded; and green is pristine) (adapted from Laurance et al., 2001). The Brazilian Legal Amazon region includes the states of Acre, Amapá, Amazonas, Pará, Rondônia, Roraima, Tocantins, and much of Mato Grosso and Maranhão.

timber being shipped anywhere in the world today is destined for China—a staggering figure! Indeed, many nations in the Asia-Pacific region and Africa now export the bulk of their timber to China (Globaltimber, 2011).

China faces three main criticisms over its role as the globally dominant timber consumer (Laurance, 2008a). First, the country has been remarkably aggressive in pursuing timber supplies globally, and in this capacity has funded or supported major new road and transportation projects to open up remote regions to exploitation, while being little preoccupied with other concerns such as social or environmental equity. Second, China has almost exclusively sought raw timber (round-logs), which is the least economically beneficial way for developing nations to exploit their timber resources. Round-logs provide only limited royalties and relatively little employment, workforce training, value-adding, or industrial development for timber-producing nations, with most of the profits being realized by foreign loggers and shippers and by wood-products manufacturers in China.

Finally, China has been a poor global citizen in attempting to combat the scourge of illegal logging (Stark & Cheung, 2006; Laurance, 2008a), which is a serious problem in many tropical nations. In some countries, one half or more of all timber entering commercial markets is effectively stolen, with no payment of government royalties and no environmental controls over cutting operations. Illegal logging is slowly diminishing globally, but this is despite, rather than because of, China's influence (Lawson & MacFaul, 2010). For this reason China is becoming increasingly vulnerable to a major boycott of its wood products, two thirds of which are exported internationally (Laurance, 2007b).

Expansion of Biofuels

Global energy demands are rising in concert with growing industrialization and living standards and an expanding human populace. A key concern is the finite nature of most high-energy-density liquid fuels, particularly petroleum and its derivatives, which power virtually the entire global transportation sector and also provide some electricity generation. At present, around 85 million barrels of petroleum are consumed each day, and many observers believe we are approaching or have reached "peak oil," the point at which available petroleum supplies inevitably begin to decline (Roberts, 2005).

With the economically realistic technologies available at present, biofuels represent the most likely alternative to petroleum. The pressure to rapidly scale up biofuel production will surely intensify as petroleum prices increase in the coming decades (and note that, because of the nonlinear nature of supply–demand curves and relative inelasticity of petroleum demand, a 5% surge in demand or a 5% decline in supply will lead to far more than a 5% increase in petroleum prices).

The expansion of biofuel production will likely be concentrated in the tropics. The tropics is where plants, which are the main feedstocks for biofuels, grow the fastest. It is also where land is typically the cheapest, certainly more so than in productive temperate regions. At least 35 million hectares of land (an area the size of Germany) is likely to be devoted to biofuel production by the year 2030, and most expansion will be in tropical countries (Sunderlin et al., 2008).

The proliferation of tropical biofuels is likely to have two negative impacts on environmental conservation (Laurance, 2008b). First, it will promote the large-scale conversion of forests and other native ecosystems for biofuel or food production to help offset the expansion of biofuel crops onto current food-producing lands. Second, by driving up competition for land and thereby elevating land prices, it will increase opportunity costs for conservation. Under such circumstances, carbon-trading will become less viable as a means to reduce deforestation and is likely to be competitive only for areas that are remote or that have poor, unproductive soils. Such areas, by virtue of their remoteness and low agricultural productivity, are the least threatened by forest conversion.

It is important to emphasize that biofuels can promote deforestation both directly and indirectly (Scharlemann & Laurance, 2008). Where they do so directly, such as when a forest is felled to produce sugarcane for bioethanol, it is obvious that that biofuel in question is poor from an environmental perspective and should, therefore, be avoided. This is because, in addition to its impacts on biodiversity and other ecosystem services, long intervals of biofuel production ranging from decades to centuries would be needed to "pay back" the atmospheric carbon emissions created by destroying the forest initially (Gibbs et al., 2008). Far more insidious, however, is that biofuels can promote deforestation in cryptic ways. For instance, generous corn subsidies in the United States designed to promote domestic biofuel production may well have promoted Amazon deforestation. This occurred when many U.S. farmers switched from producing soy to corn, causing a spike in global soy prices and a subsequent increase in deforestation in soy-producing states in the Amazon (Laurance, 2007c).

Human Population Growth

The United Nations projects that by the end of this century, the Earth will have between 9.6 and 12.3 billion people, with population growth continuing even further beyond this (Gerland et al., 2014). The human populace reached the seven billion mark in 2011 (Engelman, 2011). In the coming decades, most of the additional three billion people will be added to the rosters of tropical nations. The only major exceptions are the Middle East and northern Africa, where high population growth will be attributable to a strong Muslim influence, and the United States, where high immigration, mostly from Latin America, is expected to be a major contributor (Population Action International [PAI], 2006).

In a fundamental sense, population growth is one of the most pervasive drivers of deforestation and environmental degradation. In our highly interconnected world, population pressures manifest themselves both locally and globally; for instance, growing international demand for wood products, paper pulp, and foodstuffs are helping to spur deforestation across much of the tropics. Furthermore, population densities and growth rates both tend to be very high in biodiversity hotspots (Cincotta et al., 2000), which contain large concentrations of species that are both locally endemic and imperiled by extinction. Among nations that contain biodiversity hotspots, population growth rates are a strong and positive predictor of deforestation rates (Jha & Bawa, 2006). Similarly, nations that have high population densities tend to have many endangered bird and mammal species (McKee et al., 2003).

With some notable exceptions (e.g., Ehrlich et al., 1997; Engelman, 2011), population issues are rarely highlighted in public discourses about environmental crises, migration issues, and domestic and international conflict. This happens in part because these issues are excruciatingly sensitive. For instance, my colleagues and I were recently labeled as "misanthropic" by a pro-logging lobby group, World Growth International. This was in response to our suggestion that Papua New Guinea, where rapid population growth is promoting both social instability and environmental degradation, should improve family planning and educational support for young women (Laurance et al., 2011b).

Emerging Pathogens

We live in an era of astonishing international mobility, one in which people and their associated goods and species are in a constant state of flux. Microbes are also being moved at an exceptional pace, as evidenced by the fact that a novel human flu virus such as H1N1 can appear suddenly in southern Mexico and, in just months, have spread globally. Natural ecosystems are also being bombarded by potential new pathogens, and the resulting "pathogen pollution" could be a far more serious threat to biodiversity than many appreciate (Cunningham et al., 2003).

Novel pathogens can arise in ecosystems by a variety of means, such as host-switching, gene-swapping with other microbes, and especially by moving into a new geographic region where they encounter an abundance of unexposed, immunologically naive hosts (Daszak et al., 2000). Some of the most dramatic animal and plant mortality events ever witnessed were caused by exotic pathogens or new disease vectors. Past examples include the mass die-off of African ungulates from rinderpest (Plowright, 1982), the global extinctions or widespread extirpations of endemic Hawaiian birds from avian malaria (Atkinson et al., 1995), and the near extirpation of *Diadema* sea urchins in the Caribbean Sea (Lessios et al., 1984). Today, in addition to taking a severe human toll in Equatorial Africa, Ebola hemorrhagic fever is burning its way across the Congo Basin, causing mass mortality of gorillas and chimpanzees (Walsh et al., 2003; Thompson, 2014), whereas white-nose disease (a fungal pathogen) has killed millions of bats in North America (Blehert et al., 2009). Although little attention has focused on emerging pathogens of plants in native forests, fungal diseases caused by exotic *Phytophthora* species are known to cause severe, localized mortality of rainforest plants globally (Uchida & Aragaki, 1991; Anderson et al., 2004).

Perhaps the most devastating of all emerging wildlife pathogens is the chytrid fungus currently driving global amphibian declines (Laurance et al., 1996; Berger et al., 1998; Skerratt et al., 2007). Around 200 frog species, mostly from tropical and subtropical regions, have been driven to global extinction or been severely reduced in numbers by the fungus (Skerratt et al., 2007). Some have argued that the chytrid is merely an opportunistic pathogen attacking amphibians weakened by some unknown environmental stressor. However, a range of genetic, immunological, and other evidence suggests far more strongly that the chytrid is an emerging pathogen of exceptional virulence (Skerratt et al., 2007; Laurance, 2008c). It has been hypothesized that the chytrid fungus originated in Africa (Weldon et al., 2004), though it is now spreading rapidly throughout the world, even into some of the most pristine ecosystems on earth.

The chytrid fungus and other well-documented emerging wildlife pathogens may be merely the tip of the iceberg. In general, we tend to have a good understanding of pathogens that affect humans and our domestic animals and important food crops. Beyond this, knowledge trails off dramatically. As molecular genetics and other techniques to detect foreign pathogens improve, we may discover that emerging diseases, largely driven by the unprecedented mobility of contemporary humanity, are one of the most serious and pervasive threats to biodiversity. On top of this, the latitudinal and elevational limits of many microbes are likely to expand under global warming (Epstein, 2001; Harvell et al., 2002), and this will further increase the impacts of foreign pathogens.

Climate Change

It goes beyond the scope of this paper to delve in much detail into climatic and atmospheric changes, but I would like to highlight two issues of particular relevance for the tropics. The first is the potentially serious impact of global warming on higher-elevation tropical species. When people think of species vulnerable to global warming, visions of high-latitude species such as polar bears and caribou tend to spring to mind. However, tropical species are often more thermally specialized (e.g., Janzen, 1967; Deutsch et al., 2008; Wright et al., 2009). A polar bear, for instance, has to tolerate huge swings in temperature during the course of the year, from frigid winters to relatively balmy summers. But in the tropics, the conditions are far more stable, allowing thermal specialists to evolve and persist. Indeed, the main axis of thermal variation in the tropics relates to elevation; on average, temperatures drop by 6°C for every 1000 m rise in elevation. For this reason, many tropical species tend to be elevational specialists, either restricted to the lowlands, to mid-elevations, or to higher elevations (Janzen, 1967; Huey, 1976; Laurance et al., 2011c). Species limited to higher elevations are geographically isolated from their high-elevation brethren, frequently allowing them to evolve into locally endemic species (Fjeldså & Lovett, 1997). If one examines maps of the global distribution of endemic species, using warmer colors for areas with many local endemics and cooler colors for areas with few endemics, the world's tropical mountains seem to be on fire.

In addition to harboring a rich, highly endemic biota, tropical mountains appear highly vulnerable to global warming. Global warming could shrink and fragment the geographic range of species adapted to cool, wet montane conditions, increasing the likelihood of extinction (Williams et al., 2003; Thomas et al., 2004). Warming will also tend to elevate the cloud base on tropical mountains, reducing moisture inputs and increasing thermal radiation and desiccation stress (Pounds et al., 1999). Species in montane habitats may also have to contend with the arrival of lower-elevation competitors, predators, and pathogens as climates warm (Epstein, 2001; Harvell et al., 2002).

Modeling studies of endemic montane vertebrates in tropical Queensland suggest that a mean temperature rise of just 2°C will be enough to drive perhaps one eighth of all species to extinction (Fig. 2). If temperatures rise by more than 6°C, the models suggest, virtually all of the montane endemics are likely to vanish (Williams et al., 2003). As illustrated by the near-extinction of the northern population of the lemuroid possum (*Hemibelideus lemuroides*), which is confined to cool montane forests above 1100 m elevation in north Queensland, brief but intense heat waves, rather than steadily rising temperatures, might be the death-knell for many tropical species (Laurance, 2009b, 2011).

Another big concern is our alarmingly weak ability to predict future climate change in the tropics. We know that conditions will generally warm but, beyond this, our capacity to project future changes for any specific locality is often exceedingly poor. This point is illustrated by a series of global circulation models (GCMs) in Figure 3, each devised by leading research teams, for South America (Vera et al., 2006). GCMs are probably our most powerful tool for projecting future climate change, and these models show a series of scenarios for projected greenhouse gas concentrations for the latter part of this century. Each model in Figure 3 was run under identical conditions, and yet they vary hugely. Some, for instance, project that the Amazon basin will only become slightly warmer, whereas others suggest temperatures in the region will rise dramatically (Vera et al., 2006). And if projections of future temperatures seem wildly variable, projected precipitation is even worse. Some of the GCMs in Figure 3 suggest the Amazon basin will become wetter in the future, whereas others are far drier. Collectively, what these models illustrate is our often appalling poor capacity to downscale from GCMs, to make local-scale projections about future tropical climates (Laurance & Useche, 2009). This is especially the case for precipitation, which is vital for the persistence of tropical ecosystems. In particular, even relatively small declines in rainfall during the dry season, when forests are most drought-stressed and vulnerable to fire, could be potentially devastating for forests (Cochrane & Laurance, 2008).

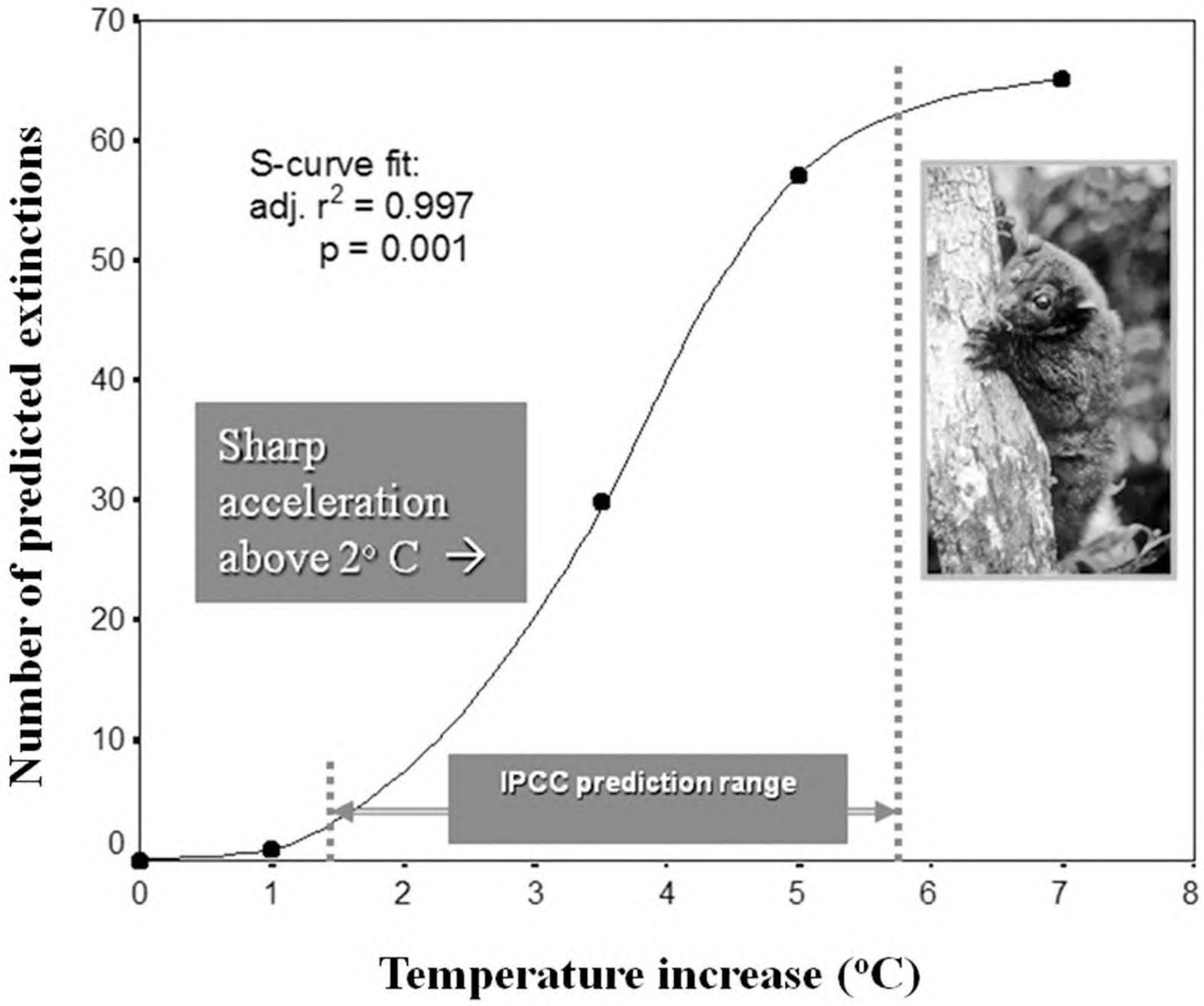


Figure 2. Projected increase in extinctions of 65 upland-endemic vertebrates in the tropical rainforests of northern Queensland as a function of future global warming (image reproduced, with permission, from S. E. Williams). Inset: a lemuroid ringtail possum (*Hemibelideus lemuroides*), which is restricted to cool, montane rainforests (photo by W. F. Laurance).

Environmental Synergisms

When speaking in public, I sometimes challenge my audiences to identify any part of the planet that is experiencing just a single environmental change. They invariably fail because no such place exists. The world is experiencing a bevy of environmental changes, from climatic change and habitat disruption to pollution and the loss of countless species, all at once. To use the analogy of a boxing match, we are not just hitting nature with an occasional jab, but with a flurry of punches landing all at once.

Such combinations of environmental threats could be the downfall for many imperiled species. For instance, an analysis of endangered and extinct mammal, bird, and amphibian species using data from the IUCN Red Data Book suggests there are far more species than expected by chance alone that are being imperiled by combinations of environmental threats, rather than by a single threat (Laurance & Useche, 2009). The tiger (*Panthera tigris*), for example, is not merely being endangered by destruction of its habitats or by widespread persecution and hunting. Rather, it is the combination of these environmental insults operating in concert that is the biggest threat to its survival. Many other synergisms—between logging and hunting (Wilkie et al., 1992), habitat fragmentation and fire (Cochrane & Laurance, 2002), and habitat disruption and climatic change (Thomas et al., 2004; Thuiller et al., 2006)—are likely to have serious impacts on tropical biodiversity.

Concluding Thoughts

I have focused here on identifying a number of emerging or potential threats to tropical forests and their biodiversity, but it is important to emphasize that "it is not all bad news." There are, in fact, many positive developments in tropical nature conservation that one could highlight. Protected areas have increased substantially in recent decades and now encompass 14.8% of the global land area (although only 5.8% is in strictly protected areas [IUCN

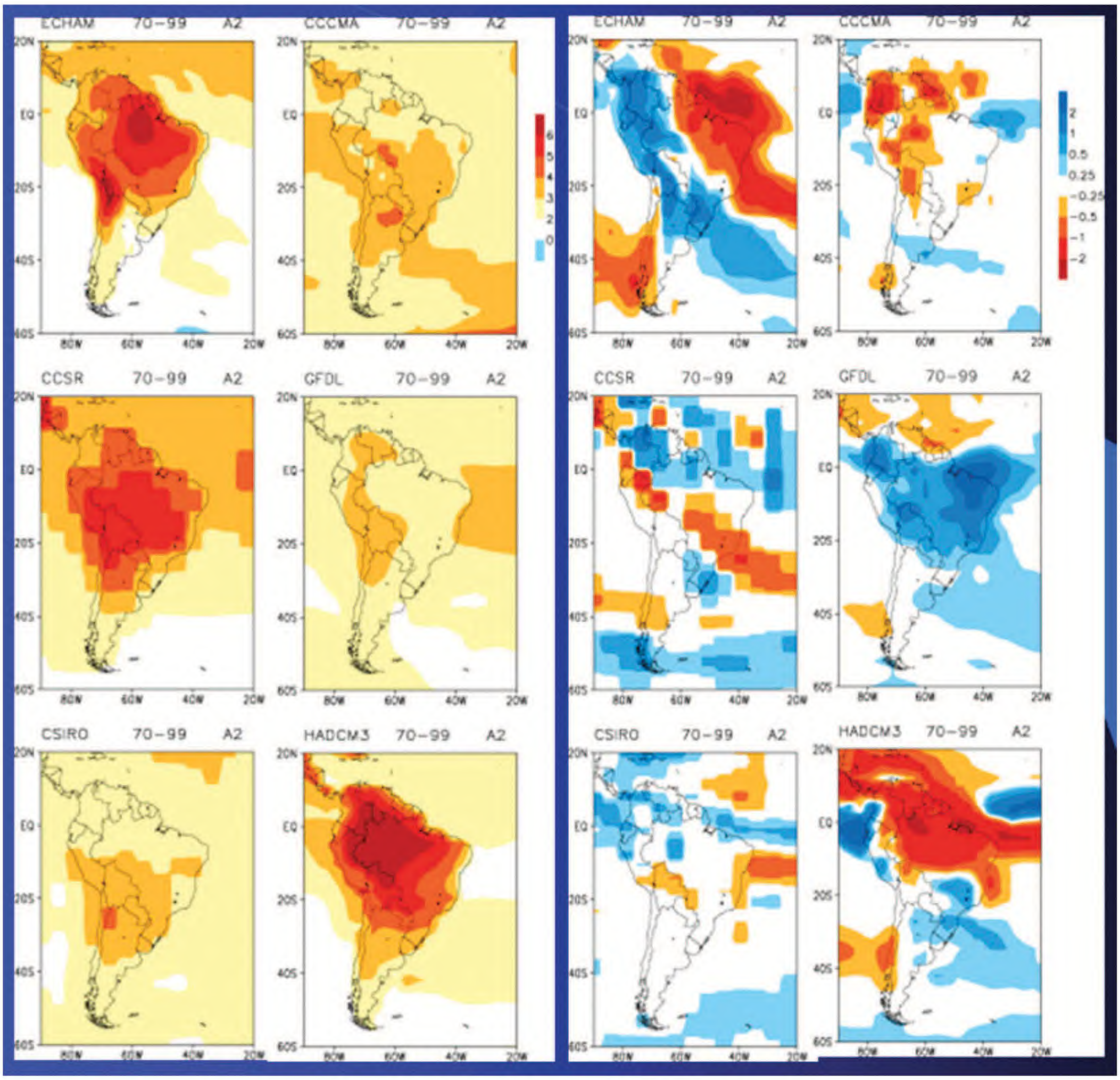


Figure 3. A variety of different global circulation models (GCMs) used to project changes in temperature (left) and precipitation (right) for South America, for the latter part of this century. All models were run under the same hypothetical conditions, using the high greenhouse-gas scenario of the Intergovernmental Panel on Climate Change (image adapted from IPCC, 2001). The vertical and horizontal axes on each model show latitude and longitude, respectively.

categories I–IV]; Jenkins & Joppa, 2009). Public awareness of the importance of tropical forests is clearly increasing (Baranzini et al., 2009), as is the number of nongovernmental groups focusing on environmental issues and indigenous peoples (Clark, 1995). International carbon-trading has emerged as a mechanism to slow tropical deforestation while simultaneously reducing greenhouse-gas emissions (Laurance, 2007d; Venter et al., 2009). While many challenges remain, conservation actions are having a positive impact on biodiversity protection at many levels (Brooks et al., 2009; Sodhi et al., 2011; Laurance, 2013).

Rather than viewing the tropics as a lost cause, one should instead view this as a time of great opportunity for tropical conservationists. Having a better understanding of the key threats to tropical ecosystems should help to guide and focus our conservation efforts and increase their effectiveness.

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HOW MANY PLANT SPECIES ARE THERE, WHERE ARE THEY, AND AT WHAT RATE ARE THEY GOING EXTINCT?

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ABSTRACT

How many flowering plant species are there? Where are they? How many are going extinct, and how fast are they doing so? Interesting in themselves, these are questions at the heart of modern conservation biology. Determining the answers will dictate where and how successfully conservation efforts will be allocated. Plants form a large taxonomic sample of biodiversity. They are important in themselves and directly determine the diversity of many other taxonomic groups. Inspired by conversations with Peter Raven, we set out to provide quantitative answers to these questions. We argue that there are 450,000 species, two thirds of which live in the tropics, a third of all species are at risk of extinction, and they are going extinct 1000 to 10,000 times the background rate. In obtaining these results, we point to the critical role of dedicated taxonomic effort and biodiversity monitoring. We will only get a good answer to the age-old question of "how many species are there?" when we understand the population biology and social behavior of taxonomists. That most missing species will be found in biodiversity hotspots reaffirms their place as the foci of extinction for decades to come. Important, but not yet addressed, are future studies of how long plant species take to become extinct in habitat fragments. These will deliver not only better estimates of extinction rates, but also the critical timeframe of how quickly one needs to act to prevent extinctions.

Key words: Biodiversity, discovery curves, extinction rates, flowering plants, missing species.

We ask four questions that arose in the context of 25 years of discussions with Peter Raven. How many plant species are there? Where are they? How many are going extinct, and how fast are they doing so? Raven has considered these questions throughout his career. Their extension to all species is obvious and, as such, they have an illustrious pedigree. For example, Westwood (1833) speculated "on the probable number of species of insects in the Creation." These questions are also both hard to answer and exceptionally rich in the subsequent explorations we must undertake to get the final answers. We argue that there are 450,000 species, two thirds of which live in the tropics, a third of all species are at risk of extinction, and they are going extinct 1000 to 10,000 times the background rate. In obtaining these results, we point to the critical role of dedicated taxonomic effort and biodiversity monitoring.

We shall consider each of the questions in turn but not in the order that we thought about them. Pimm and Raven (2000), in a commentary that accompanied Myers et al. (2000), addressed the last question first by building simple scenarios of extinction based on the loss of tropical forests. It quickly became apparent that the answer would depend on how many species lived in tropical forests and, indeed, in which particular forests. In turn, that meant knowing not only how many species were known, but also how many were still unknown to science. We start with those questions.

HOW MANY PLANT SPECIES ARE THERE?

There are two questions in estimating the total numbers of plant species: how many do we know, and how many do we not know, i.e., how many are still unknown to science? For shorthand, we will call the latter "missing species." The first question might seem easy. The problem of synonymy (i.e., taxonomists giving different names to the same species inadvertently) complicates it. There have been several recent estimates of the currently known number of unique species of seed plants (Prance et al., 2000; Govaerts, 2001; Bramwell, 2002; Thorne, 2002; Scotland & Wortley, 2003; Paton et al., 2008), with the highest estimate (422,127; Govaerts, 2001) two times the lowest one (223,300; Scotland & Wortley, 2003).

The Plant List (<www.theplantlist.org>) is an ongoing collaboration between the Missouri Botanical Garden and the Royal Botanic Gardens, Kew. In May 2013, it estimated that there were 352,000 species of flowering plants (angiosperms; the same number as Paton et al., 2008), with 298,900 accepted names and 263,925 names yet to be assessed, though surely many of them will be synonyms. Because the

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accepted names among those resolved is 38%, it seems reasonable to predict that the same proportion of unresolved names will eventually be accepted. This yields another ca. 100,000 species for a total estimate of ca. 400,000 species. These are monumental assessments, necessary because the individual names, identities, and relationships are the basis of so much subsequent science.

Yet, there are also questions that require estimating how incomplete this catalogue is. How much more effort will be required to complete it? Are we years, decades, or centuries away from a widely accepted answer? For consortia such as the Catalogue of Life (<http://www.catalogueoflife.org>), these are fundamental questions. At a local scale, projects such as the All Taxa Biodiversity Inventory ask how many species live in an area. For the Great Smoky Mountains National Park in the eastern United States, the answer is 7636 of all species combined (as of 5 October 2014 there are 7799 species, 931 of them new to science), with 923 of these (13%) new to science (<http://www.dlia.org/node/204>). That so many species of animals and plants are new in an otherwise well-explored area surrounded by major research universities is a testament to our ignorance about life's diversity.

There are practical concerns, too, about knowing the number of missing species. Because they are most likely rare, their number and geographical distribution are essential to answering the second and third questions we ask.

There have been many previous attempts using, e.g., scaling laws in food webs, abundance, body size, and rarity to estimate the number of missing species (May, 1988, 1990, 1992). Recent attempts employ what surely seems like the most promising approach. As the pool of missing species declines, the numbers of discoveries will also likely decline in direct proportion (Solow & Smith, 2005; Wilson & Costello, 2005). The obvious analogy is with predator-prey interactions: taxonomists are the "predators," and as the supply of "prey" (i.e., missing species) declines, so too should the capture rate.

TAXONOMISTS ARE PREDATORS, MISSING SPECIES ARE PREY

Completely counter to this expectation, the numbers of both monocot and non-monocot species described per five-year interval have increased almost exponentially since 1950 (Joppa et al., 2011b). To take an example, since 1950, there have been 95 accepted species names for Onagraceae. From 1900 to 1949, taxonomists named only 43 species.

Moreover, a broad exponential increase in names applies to other taxa including mammals, spiders, amphibians, and example genera of marine gastropods (Joppa et al., 2011c). Under such circumstances, estimates of missing species from extrapolations of uncorrected discovery rates will be nonsense. (Only birds are exceptional and for which the taxonomic catalogue now appears almost complete.)

The underlying problem is that the total number of plant taxonomists active in any period has increased exponentially, too, doubling about every 30 years. (We define "taxonomists," simply as those who describe new species.) Broadly similar rates characterize the animal taxa. Again, excepting birds, the rates of increase are even higher over the last half century. Given this observation, it is not particularly surprising that the raw numbers of species described over time have increased as well. The number of taxonomists is a powerful predictor of the number of species described.

The solution to this problem also seems obvious: divide the number of species described by the total number of taxonomists and expect that this ratio will decline as the pool of missing species declines. This also fails.

The World Checklist of Selected Plant Families (WCSP; <http://www.kew.org/wcsp>) provides a sample of approximately 119,000 species of systematically revised species, including all of the monocots and selected non-monocot families. We show that for both monocots and selected non-monocot families, there was an increase in the number of species described per taxonomist, typically for the first century or so after Linnaeus (Joppa et al., 2011b). Taxonomists have likely increased the efficiency of their efforts since the mid 1700s when Linnaeus introduced the system of binomial nomenclature and founded modern taxonomic practice. There could be many reasons for taxonomists becoming more efficient over time, of course. Clearly, the world is easier to explore now than it was in the past; natural history collections are more accessible; techniques are better; there is more collaboration, and so on. In the predation analogy, the predators have become smarter over time.

Combining these considerations suggests a model where one predicts the numbers of species described per taxonomist over a given interval in terms of the continually diminishing pool of missing species, combined with some simple increase in the efficiency of the taxonomic enterprise. By doing so, we concluded that between 10% and 20% of the current species total are missing, suggesting that there are

probably about 450,000 species of flowering plants (Joppa et al., 2011b).

We also estimated the numbers of missing species family by family to assess which families might hold the greatest number (Joppa et al., 2011b). Then, we compared our estimates to those based on a selection of taxonomic experts who knew the families. There was broad agreement between the models and the experts.

Now, the details of what constitutes a taxonomist and who contributes what to a species description are elsewhere (Joppa et al., 2011c), but the Onagraceae are broadly typical of other families. In the century after Linnaeus (1753), 74 taxonomists described 141 Onagraceae species and mostly did so on their own. Since 2000, 34 taxonomists have contributed to descriptions and revisions of 47 species of Onagraceae. Single authors described only four of these species; the rest were collaborations. Collaborators are not mere assistants, however, but those who, at least eventually, describe species in their own right. Of those 34 recent taxonomists, 25 of them were senior authors on one or more descriptions. Joppa et al. (2011c) also show that taxonomists are becoming more specialized over time. The modern tendency is for taxonomists to work on only one or a few families and often in one broad geographic region. In the predation analogy, predatory taxonomists are now more social and more specialized than they were before.

For some plant families (e.g., Onagraceae), the number of species described per taxonomist does not obviously drop over time. This defies any effort to predict the number of missing species from simple description rates. Inspection of the descriptions shows that taxonomists (in this case, often Raven and his colleagues) are not "eating" species randomly but working their way through plant families, group by group. That is, taxonomists revise a family genus by genus, doing so at a rate that reflects the resources and time at their disposal. Genuinely new species as well as revisions of old ones appear in clusters by group, sometimes a few at a time, sometimes involving many. When the supply of unrevised genera dries up, we can expect the rates of description to drop dramatically.

We have yet to model this circumstance. It is clear that as we delve deeper into particular plant families, the complex social behavior and working habits of taxonomists become more important in driving the rates of species' descriptions. Nonetheless, for large sets of species (e.g., all monocots), the basic model of taxonomists as predators gives a good statistical description of how many missing species receive names each year.

Where Are the Missing Species?

Obviously, we can apply these "taxonomists as predators" models to predicting *where* missing species live. The WCSP gives species locations based on classifying the world into 369 regions. Only 33 of these ranges have sufficient endemics to run our models, but by judiciously combining regions, we grouped about 72% of the species in 50 broad areas (Joppa et al., 2011a). For example, many countries in Central America are quite small and individually have few endemics. Yet, a combined 9% of all known species occur there. The remaining 28% of species occur in two or more of the regions as we defined them.

So, where is plant diversity highest once we adjust for where we think the missing species occur? Our analyses leave untouched the idea that plant species are mostly tropical; almost exactly two thirds (65.7%) of all species, known and missing, live in the tropics (Joppa et al., 2011a). That is exactly the fraction that Raven (1981) asserted were tropical.

We predicted that the great majority of the missing species would be in the region from Mexico to Panama (6% of all the predicted missing species), Colombia (6%), Ecuador to Peru (29%), Paraguay, Argentina, and Chile (5%), southern Africa (16%), and Australia (8%). These areas combined have 70% of all the species we predict are missing. All of these areas are biodiversity hotspots (Myers et al., 2000), which leads directly to our next question.

How Many Plant Species Are Going Extinct?

Estimating the fraction of plant species threatened with extinction requires that we understand how many species are missing from the taxonomic catalogue (Joppa et al., 2011b). Brummitt et al. (2008) suggested that 20% of known plant species are threatened. Take this estimate, and then add to that our result that there are 10%–20% more missing species. Then assume that essentially all of these missing species have small geographical ranges and are locally rare. That is likely why they are missing, after all! We predict that they are in the biodiversity hotspots that, by definition, have high levels of habitat loss. Thus, the missing species are also surely threatened with extinction. This argument predicts that 27%–33% of all plant species are likely threatened. These estimates are based on immediate threats and do not consider further development of

destructive factors, including climate disruption (Pimm, 2009), during the remainder of this century.

So, how good are the assumptions that the missing species have small ranges and that taxonomists will indeed find them in the biodiversity hotspots? Understanding where missing species are likely to live is vital in setting international priorities for conservation and ensuring that we find and protect the missing species before we drive them to extinction.

Answering this question requires several of the basic "laws" of biodiversity. By "law" we mean generalizations or patterns, if you will, that apply widely and across many taxa. The first law is that *the average geographical range size of a group of species is very much larger than the median range*. For vertebrate taxa, there are now global maps of species' ranges. The average of the geographical ranges of 1684 species of mammals in the New World is 1.8 million km^2, but 50% of those species have ranges smaller than 250,000 km^2, a seven to one ratio. For the region's three main bird groups (non-passerines, suboscine passerines, and oscine passerines), the ratios are between five and eight to one. For amphibians, they are 40 to one. Simply, there are many species with small ranges and few with large ranges.

We do not have comparable data for flowering plants, unfortunately. In the sample of systematically revised species compiled from the WCSP database (Joppa et al., 2011a), one half of the species occur in regions that individually are no larger than 1.4 million km^2. Of course, these species do not occur over the entire extent of the region in which they occur. Moreover, many other regions contain small-ranged species. Therefore, 1.4 million km^2 sets a high upper limit for the median range size of a flowering plant. There are surely many plant species with small ranges.

The next law of biodiversity is that *species with small ranges are geographically extraordinarily concentrated*. In total, 88 of the 369 regions covering only 5% of the ice-free land surface combined hold 30% of the known plant species, while 111 regions covering a combined total of 10% hold 40% of the species.

Currently, small geographical range is overwhelmingly the best statistical predictor of whether a species will be in danger of extinction. Indeed, sufficiently small geographical range combined with an occurrence in areas of extensive habitat loss classifies a species as being "threatened" under the IUCN Red List criteria (<http://www.iucnredlist.org/static/categories_criteria_3_1>). Understanding extinction rates requires knowing whether the areas where species with small ranges are concentrated coincide with places where there is extensive habitat loss.

The unique contribution to conservation from Myers et al. (2000) was to note not only that small-ranged species are geographically concentrated, but also that perversely most are located in areas with disproportionately high levels of habitat destruction. Cincotta et al. (2000) added high human population growth to the list of threats to these biodiversity hotspots.

Given that we have shown the taxonomic catalogue is short by 10%–20%, then how would an understanding of where the missing species live alter our views of hotspots? For obvious reasons, new species discoveries are overwhelmingly of species that have small geographical ranges. Will knowing where the missing species reside change the way we set conservation priorities? Will relative priorities change as taxonomists complete the catalogue? Will new priorities become apparent? Are the missing species in places where they are likely to be threatened? Will we discover them before they become extinct?

We have already anticipated the answers to these questions: the hotspots hold the majority of the species we predict to be missing. The missing species are in harm's way and possess small ranges. Myers et al. (2000) concluded that the hotspots are where species are going extinct. Our estimates of where the missing species occur do not contradict this conclusion (Joppa et al., 2011a). What the estimates do show is that extinctions are even more likely to be concentrated in hotspots than we previously thought.

Are there some places where recent habitat loss might cause the region to now qualify under criteria from Myers et al. (2000) as a hotspot? New Guinea, e.g., has far greater than 1500 endemic plant species but was not originally included as a hotspot due to relatively intact natural vegetation. Ongoing loss of natural habitat through logging, mining, and road construction (Shearman et al., 2009) could make the region a likely candidate for inclusion as a hotspot in the near future. Increased access could also lead to an increase in the descriptions of new species, of course. New Guinea's inaccessibility may explain its low rates of description and the predictions of there being few missing species.

EXTINCTION SCENARIOS

Pimm and Raven (2000) used results from Myers et al. (2000) to develop scenarios of how many species would become extinct. We used the familiar

species-area relationship ($S = cA^z$) that relates the number of species (S) to area (A), using two parameters, c and z, for which z is vital. The parameter c is a constant of proportionality. For samples of different sizes within continuous habitat, z is very small (Rosenzweig, 1995). This describes, e.g., the observation that the number of tree species in the Great Smoky National Park is not that much smaller than the total number across eastern deciduous forest from Florida to Maine and westward to the prairies.

Now, what happens after habitat destruction? Two things: first, some species will go extinct immediately, because they lived only in the destroyed area. Metaphorically, they go extinct "overnight." The second wave of extinction invokes a separate species to area relationship, that for oceanic islands where the areas involved are isolated. Here, z is much larger, typically about one fourth (Rosenzweig, 1995), meaning in simple terms, an island one half the size of a larger one will have 85% as many species.

The difference between the two relationships comes from Preston's (1962) classic work. He understood that for isolated populations there is a minimum viable population size. Suppose we reduce the total number of individuals by one half, shrinking as it were the larger island to the smaller one. Then 15% of the species would have population sizes too small to be viable. After habitat loss, some species will go extinct "overnight," but many more will linger until chance events eventually doom them. Such experiences fit well with much practical experience of endangered species. In addition, it fits with what we know of the risks from stochastic events and inbreeding in small populations (Brooks et al., 2011).

There are now many calibrations of forest losses and subsequent extinctions, at least for birds and mammals, the taxa that we know best. Pimm and Askins (1995) asked how many bird species we expect to go extinct following the loss of forests in eastern North America. About 150 species lived in those forests, but only 30 of these were endemic to those forests. Pimm and Askins applied the island species-area relationship (because by 1870 eastern North America was a series of forest islands) to the 30 endemic species, because the other species survived in Canada and elsewhere in North America. This model predicts four and one-half extinctions. As forests were cleared in the 18th and 19th centuries, there were four documented forest bird extinctions, and one species became endangered.

Many other studies find similar, compelling agreements between forest losses and either the number of actual extinctions or the number of endangered species (Brooks et al., 1997, 1999a; Cowlishaw, 1999; Brook et al., 2003). Armed with this agreement, Pimm and Raven (2000) took the estimates of endemic species in the hotspots Myers et al. (2000) defined, with the estimates of remaining habitat and, thus, predicted the total extinctions that would occur. For example, Myers et al. estimated that only 7.5% of the Atlantic coast rainforest of Brazil remains and that this area contained 2.7% of their estimate of 300,000 plants as endemics to this area. The continued rate of species descriptions should eventually yield 3.6% of 450,000 species of plants (Joppa et al., 2011a) for this area. Applying the species-area calculation, about one half of the endemic plant species in this region should be endangered (ca. 4000 according to Myers et al., but 7200 with the missing species included).

This and similar calculations have uncertainties, of course. This example suggests that the largest uncertainty is surely that the estimate of the missing species comes from present rates of species descriptions. Those rates now reflect collecting and systematic revisions of plants from a mere 7.5% of the region's original forested area. There is a strong possibility that there may have been many extinctions across this area that are not estimated by our "taxonomists as predator" models of species discovery. Simply, there are the species we know, some of which are threatened and some of which are not. Second, there are the species we estimate are missing from the taxonomic catalogue. We argue above that surely all of these are threatened. There is a final class of species: those that went extinct before we could even estimate that they were missing from the taxonomic catalogue.

Most of the remaining Atlantic Coast forest habitat is in mountains. Almost all of the lowland forests are gone. How many species occurred in the lowland forests before they were destroyed is beyond our present ability to model. What we know of the region's orchids is that one half of the records are from only one location and a quarter of the 600 species in the state of Espírito Santo (ca. 46,000 km^2) are endemic to it (Pimm, 2005). Almost certainly, the median range size of orchids (at least) was so small that many species could have lived in areas completely destroyed before taxonomists explored them.

HOW LONG DOES EXTINCTION TAKE?

Apart from the small fraction of species that go extinct "overnight" after habitat loss, most species linger, declining inexorably toward extinction. The obvious question is how long does this take? For the

birds in eastern North America, we know it took decades after the low point of forest cover in 1870 before the species finally expired. Pimm and Raven (2000) assumed 50 years for the half-life. This affirms the biodiversity hotspots as the foci of extinction for decades to come. Studies of how long plant species take to be lost from habitat fragments that parallel those for birds (Brooks et al., 1999b; Ferraz et al., 2003) are vital, not only to get better estimates of extinction rates, but also to give an idea of how quickly one needs to act to prevent extinctions.

The background rate of plant and animal extinctions—those before human impacts—appear to be about one extinction per ten million species per year (de Vos et al., 2014). Amphibians, birds, and mammals have current extinction rates of approximately 100 extinctions per million species per year, (that is, 1000 times faster). If currently threatened species last a century, a most optimistic scenario, the extinction rates will be ten times higher. Plants have a higher fraction of threatened species than do these vertebrates so the plant extinction rates are likely to be comparable or higher.

Pimm and Raven (2000) considered the dynamics of unfolding habitat losses only briefly. They considered two scenarios: extinctions based on how much habitat remained in 1990 within the biodiversity hotspots and how much habitat was protected, assuming that only this area would remain intact. Since then, there have been new threats to species in the form of oil-palm cultivation, especially in Southeast Asia, but also encouraging signs in the increase of the global total of protected areas (Jenkins & Joppa, 2009) and the success of protected areas in reducing habitat loss (Joppa et al., 2008; Joppa & Pfaff, 2011). Exactly where protected areas are established is a key variable in driving extinction rates (Rodrigues et al., 2004), and many protected areas are not in the necessary places (Joppa & Pfaff, 2009).

Finally, Pimm and Raven (2000) did not consider global change. Given our predictions that the greatest numbers of threatened plant species are likely to be the northern Andes, the coastal forest of Brazil, Mexico, and Central America, and other mountainous areas, this raises an obvious question. Because the latitudinal gradient of temperatures in the tropics is weak, the way species will avoid the effects of climate disruption will be to move to higher elevations (Pimm, 2009). That almost inevitably means less suitable areas for species, some of which may simply run out of space as their ranges move higher into the mountains, if they can get there in the first place (Forero-Medina et al., 2011). These species are likely to be separate from those species threatened by habitat loss, making climate disruption an added and potentially significant extra cause of extinctions.

Discussion: What We Need to Know

Asking how many species there are suggests that we will only get a good answer when we understand the population biology and social behavior of taxonomists (Joppa et al., 2011c). We cannot understand where species live unless we know where the missing species occur. Likely, we also need to know where species have already become extinct before taxonomists started their explorations and classifications. How fast species become extinct requires knowledge of how long species last in the habitat fragments that are presently their only homes. It also requires an understanding of how many species live in tropical mountains too close for comfort to the mountain tops given minimum projected global temperature rises of 2°C.

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EVOLUTION OF ANGIOSPERM POLLEN. 1. INTRODUCTION[1]

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Abstract

This paper is the first in a series that documents the diversity, distribution, and evolution of palynological characters across angiosperms in a contemporary phylogenetic context, using modern optimization methods. The objectives of the series are: (1) to describe the diversity of pollen morphologies across the angiosperms; (2) to estimate ancestral palynological character states, diagnostic characters, and synapomorphies for monophyletic groups; (3) to highlight and interpret inferred patterns and processes of evolution in palynological characters; and (4) to provide a framework for the placement of enigmatic taxa (including fossil taxa) based on pollen morphology. This first paper examines the methods available for such a study and presents an overview of palynological characters across angiosperms as a whole. Using a well-supported, recent, molecular phylogeny, we consider the effects of coding strategy, method of optimization, and starting tree topology upon inference of trait evolution. Coding strategy and optimization method had significant effects upon inferred ancestral character states, the latter probably due to the different evolutionary models applied. Phylogenetic topology had little effect upon inferred ancestral character states, because the uncertainty in topology at this level involved only nodes where few character state changes occurred. Several palynological characters showed consistent, structured patterns in the context of phylogeny: angiosperms are distinguished from other seed plants by character states including supratectal elements echinate and less than 1 μm in size, and infratectum structure columellate; eudicots, as recognized in previous studies, may be defined by globose, isopolar, radially symmetrical grains with three equatorial apertures. We present a framework for the remainder of the series, in which the angiosperms are divided into nine monophyletic and paraphyletic groups each having a similar level of pollen variability, and a set of recommendations for the analysis of these groups. The series will provide a reference for future palynological and systematic studies and an approach that may be replicated for other character sets.

Key words: Ancestral character states, diagnostic characters, evolution, flowering plants, optimization, palynology, phylogeny, synapomorphy, trait evolution.

There is a long and illustrious history of studying palynological characters in a broad evolutionary context (e.g., Wodehouse, 1935; Erdtman, 1943, 1944, 1945, 1946, 1948, 1952; Walker & Doyle, 1975; Nowicke & Skvarla, 1979). The remarkable potential of palynological characters in systematic classification, recently discussed by Blackmore (2000), lies in the fact that pollen grains contain a remarkable degree of information for their minute size, which is in turn due to a number of factors including their highly resistant (almost indestructible) sporopollenin wall, great abundance, and incredible, heritable, variety of form (Blackmore, 2007). The importance of pollen characters in systematics has been highlighted through the recognition of a monophyletic tricolpate clade within angiosperms (Donoghue & Doyle, 1989), now widely known as the eudicots (e.g., Doyle & Hotton, 1991; APG, 1998; APG II, 2003; APG III, 2009). It is likely that further synapomorphic palynological characters remain to be discovered through our growing understanding of both plant phylogeny and pollen development and structure.

The fact that pollen characters are both variable and characteristic of taxa was perhaps first noted by Grew (1682). However, it was more than a century later that Brown (1811) recognized they might therefore be useful for classification (Fig. 1A). The first attempt at a full classification of the flowering plants based on pollen grains was made by von Mohl (1835; Fig. 1B). Von Mohl's work seems to have been largely overlooked until it was rediscovered by

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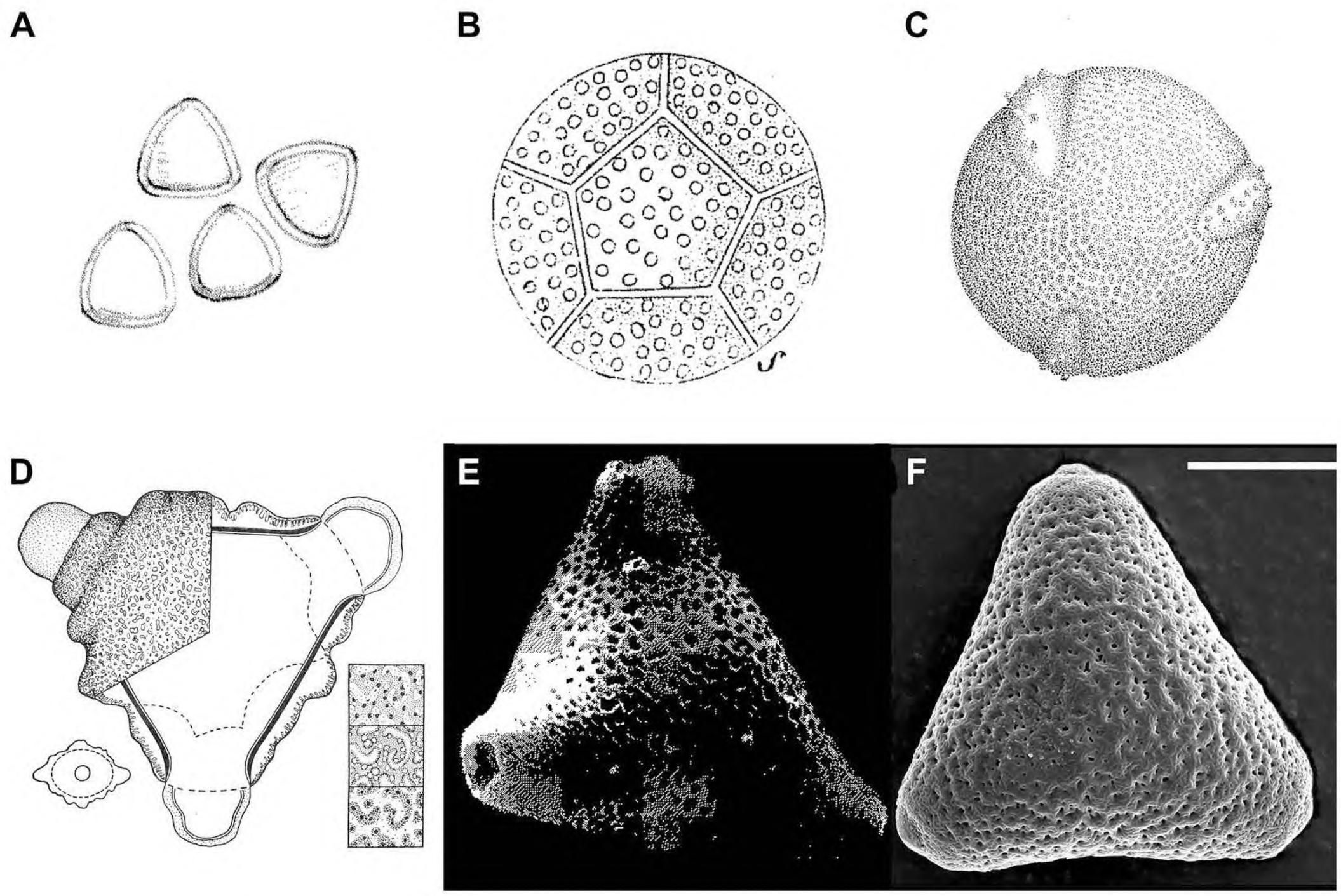


Figure 1. Selected images from the history of palynological study, using examples from the Proteaceae and other basal eudicot groups. —A. *Knightia excelsa* R. Br. (Proteaceae), "pollen plurimum auctum" (mature pollen), reproduced from fig. 7 of Brown's 1811 thesis, *On the Proteaceae of Jussieu*. —B. *Fumaria spicata* L. (Papaveraceae), "graine mouille" (hydrated grain), reproduced from fig. 24 of von Mohl's 1835 work, "Sur la structure et les formes des graines de pollen." —C. *Platanus occidentalis* L. (Platanaceae), polar view, reproduced from plate IX, fig. 5, of Wodehouse's 1935 work, *Pollen Grains: Their Structure, Identification and Significance in Science and Medicine*. —D. *Grevillea bipinnatifida* R. Br. (Proteaceae), fig. 208, reproduced with permission of the Palynological Laboratory, Swedish Museum of Natural History, Stockholm, from the 1972 corrected reprint edition of Erdtman's 1952 epic, *Pollen Morphology and Plant Taxonomy*. —E. *Euplassa inaequalis* Engl. (Proteaceae), triporate pollen, polar view, an early SEM micrograph (fig. 3B) reproduced with kind permission of Missouri Botanical Garden Press from Walker and Doyle's 1975 study, "The bases of angiosperm phylogeny: Palynology." —F. *Sorocephalus crassifolius* Hutch. (Proteaceae), whole grain, polar view, reproduced with kind permission of the American Society for Plant Taxonomy from fig. 15A of Sauquet and Cantrill's 2007 comprehensive evolutionary study, "Pollen diversity and evolution in Proteoideae (Proteales: Proteaceae)."

Wodehouse (1935). Wodehouse himself published a series of beautifully illustrated works on the structure of pollen grains and their use in classification (e.g., Fig. 1C), focusing on Asteraceae (Wodehouse, 1926, 1928a, 1928c, 1929a, 1929b, 1930, 1936). Shortly after this, Erdtman (1943) produced the first of his many surveys of pollen and spores throughout the land plants. His work, *Pollen Morphology and Plant Taxonomy* (Erdtman, 1952), remains the most comprehensive reference guide to angiosperm pollen. It not only describes and illustrates pollen grain structure in great detail (e.g., Fig. 1D), but also draws parallels between families based on palynological characteristics. Sadly, only a small proportion of Erdtman's work has been published, with descriptions and illustrations of a great range of taxa remaining only in the archives of the Swedish Museum of Natural History in Stockholm.

The 1970s saw a gradual progression from simple descriptions of pollen morphology to hypotheses of evolutionary change in pollen characters. Muller (1970) published an evolutionary scenario showing relationships between pollen grain types linked to plant evolution as then understood. Walker and Doyle (1975), using some of the first SEM micrographs (e.g., Fig. 1E), continued in this evolutionary vein at the Bases of Angiosperm Phylogeny symposium (Walker, 1975) at the 24th annual meeting of the American Society of Plant Taxonomists, presenting similar diagrams to Muller (1970) and highlighting evolutionary trends correlated with relative advancement of taxa for a wide range of pollen morphological characters (Walker & Doyle, 1975). Later, at the Evolutionary Significance of the Exine meeting (Ferguson & Muller, 1976), Van Campo (1976) explored trends of palynological evolution in

various angiosperm taxa. Shortly afterward, Nowicke and Skvarla (1979) published a study of pollen morphology and development across 650 species focusing on the Centrospermae (roughly corresponding to today's Caryophyllales). Their study determined diagnostic characters for higher-order groupings, albeit without explicit reference to phylogenetic relationships (Nowicke & Skvarla, 1979). These evolutionary approaches were complemented by Muller's ongoing documentation of angiosperm pollen in the fossil record (Muller, 1970, 1981, 1984). In sum, advances in phylogenetic, palynological, and paleobotanical research, including the relatively new techniques of SEM and transmission electron microscopy (TEM), provided a solid foundation for future interpretations of pollen and spore morphology in a phylogenetic and ontogenetic context, provided by concurrent progress in the visualization of development and numerical (cladistic) methods for phylogenetic analysis (for discussion see Funk & Stuessy, 1978).

Together, these precipitated in the early 1980s a burst of cladistic analyses of pollen characters (alone or in combination with other morphological characters) at familial and lower levels. Early examples include the works of Blackmore (1982) on the Scorzonerinae (Asteraceae), Lee and Park (1982; Oleaceae), Donoghue (1983; *Viburnum* L. [Caprifoliaceae]), Kress and Stone (1983; Heliconiaceae), Blackmore and Cannon (1983; Morinaceae), and Linder (1984; Restionaceae; see Appendix 1 for more recent examples). In such studies, morphological character states distributed across groups in a phylogeny are interpreted as homoplasies, synapomorphies, autapomorphies, or plesiomorphies, all of which may also be diagnostic for the purposes of classification. These concepts enable the generation of evolutionary hypotheses regarding ancestral and derived states and the direction and pattern of evolution in the observed traits. It may also be possible to identify correlations between the evolution of palynological character states, life history strategies and syndromes, and evolutionary-ecological factors such as the origin or diversification of particular pollinating animal groups.

Notwithstanding the conceptual and factual advance represented by the first cladistic studies, they were limited in two respects. Firstly, these early studies typically tended to combine the use of palynological characters as evidence for the reconstruction of phylogenetic relationships with their interpretation in the context of the resultant phylogeny (for an early exception see Jansen et al., 1991). In the absence of an independent phylogenetic estimate, for instance based on molecular genetic data, the interpretation of characters as ancestral or derived, synapomorphy or homoplasy can thus be seen as a circular argument (for discussion see de Queiroz, 1996); others argue that recognition of characters as synapomorphic or homoplastic is intrinsically, and rightly, bound up in the process of phylogenetic estimation (most famously Patterson, 1982). Secondly, perhaps due to the large amount of data required, analysis has been limited to the family level and below. Even had it been possible to explore further in the taxonomic hierarchy, the results would have been erroneous because understanding of angiosperm phylogeny at the time (e.g., Takhtajan, 1980; Cronquist, 1981; Dahlgren, 1988; Thorne, 1992) differed considerably from present-day interpretations. For instance, Walker and Doyle (1975) utilized the broadly phenetic classifications of Cronquist (1988) and Takhtajan (1980), which significantly influenced their inference of trait evolution.

Since the 1990s, there has been a concerted shift toward classifications that prioritize monophyly. For flowering plants, the widely accepted Angiosperm Phylogeny Group (APG) classifications are based largely on molecular phylogenetic studies conducted over the last two decades (APG, 1998; APG II, 2003; APG III, 2009). The first APG classification (APG, 1998) recognized 462 families in 40 putatively monophyletic orders and a few higher groups. Five years on, subsequent changes in family circumscription and ordinal definition were incorporated (APG II, 2003), giving a classification with 457 families in 45 orders. The most recent edition (APG III, 2009) consolidates these into 415 families among an increased 58 orders. Overall, the APG classifications have proven extremely stable. In the most recent, the authors note that "we do not see the APG classification as continuing to mutate for the indefinite future. . . . We hope the classification . . . will not need much further change" (APG III, 2009: 106).

Enabled by such advances in phylogenetic classification, pollen development, electron microscopy (Blackmore, 2007), and methods for optimization of character states upon phylogenies (e.g., Cunningham et al., 1998; Mooers & Schluter, 1999; Omland, 1999; Pagel, 1999a, 1999b; Huelsenbeck et al., 2000; Huelsenbeck & Bollback, 2001; Nielsen, 2002; Pagel et al., 2004; Ronquist, 2004; Vanderpoorten & Goffinet, 2006; Ekman et al., 2008), we can now re-evaluate the evolution of palynological characters across the flowering plants within a more explicit phylogenetic, evolutionary, and ontogenetic context. Similar angiosperm-wide studies have suc-

cessfully been carried out on the evolution of floral symmetry (Jabbour et al., 2009) and secondary chemicals (Esteban et al., 2009). For palynological characters, evolutionary studies have been conducted in the Proteaceae (Sauquet & Cantrill, 2007, an SEM micrograph from which is shown in Fig. 1F) and Asteraceae (Blackmore et al., 2009) which, with ca. 20,000 species (Funk et al., 2005), comprises 7%–10% of flowering plants. Both Sauquet and Cantrill (2007) and Blackmore et al. (2009) demonstrated that in the context of robust phylogenies, pollen characters can provide diagnostic and synapomorphic characters at a range of hierarchical levels, enabling the formulation of adaptive hypotheses. Furthermore, the recovery of ancestral character state combinations may better enable the placement of fossil pollen grains in a phylogenetic context, facilitating greater understanding of the date and location of key events in angiosperm evolution.

In a series of papers, of which this is the first, we will survey the evolution of pollen morphologies throughout angiosperms, based on the most recent and reliable phylogenetic trees and incorporating an extensive literature review and new palynological data. Our objectives are: (1) to describe and document the diversity of pollen morphologies across the angiosperms; (2) to estimate ancestral palynological character states for monophyletic groups and thereby to identify diagnostic characters and synapomorphies; (3) to highlight and interpret inferred patterns and processes of evolution in palynological characters; and (4) to provide a framework for the phylogenetic placement of enigmatic taxa based on pollen morphology (this is especially relevant to extinct taxa since pollen is often particularly well preserved in the fossil record). This first paper compares the available methods for investigating trait evolution in a phylogenetic context and provides an overview analysis of angiosperm palynological characters and their evolution at a broad scale. We hope this will stimulate similar review and analysis of other classes of morphological and anatomical characters.

The Influence of Methodological Factors in the Analysis of Trait Evolution

Three fundamental methodological factors may influence the inference of ancestral morphological character states upon phylogenetic trees: coding strategy, analytical (optimization) method, and starting tree topology (i.e., phylogenetic uncertainty).

Coding morphological characters becomes nontrivial for higher (supraspecific) taxa when more than one character state is found therein. Three main coding strategies are recognized in phylogeny reconstruction; these may equally be applicable to studies of trait evolution. The ancestral (sensu Bininda-Emonds et al., 1998) or groundplan (sensu Yeates, 1995) method uses fossils, ontogenetic data, or prior phylogenetic analysis to estimate a primitive state in each monophyletic higher taxon. The democratic (Bininda-Emonds et al., 1998) or composite (Brusatte, 2010) method uses data from a sample of taxa within each higher taxon, which is then represented by the most common state found (based on the hypothesis that "common equals primitive"). The exemplar method (Bininda-Emonds et al., 1998) employs one or more representatives of each higher taxon, chosen either for their inferred plesiomorphic status or simply their availability. A fourth possibility, to code higher taxa as polymorphic, is not usually considered suitable for reconstruction of phylogenetic trees but is, however, open to character studies based upon existing phylogenies. Like the democratic method, it uses a sample of taxa from across the higher taxon but does not assume that common equals primitive; the higher taxon is coded for *all* states found in its representatives. We term this the comprehensive method.

The choice of analytical methods for character state optimization has received less attention in the literature than that for reconstructing phylogenies (Pagel, 1997; Cunningham et al., 1998), and their implementation remains controversial (Yang, 2006; Wiens et al., 2007). As with phylogeny reconstruction, both parsimony and model-based methods exist. Maximum parsimony (MP), the most widely used method (Cunningham et al., 1998), estimates ancestral states so as to minimize the number of state changes implied across the phylogeny as a whole. This method has proven informative and further has the advantage that it is insensitive to the density of taxon sampling within a clade for which a trait is fixed (D. Barker, 2011, pers. comm.). However, MP by definition assumes that changes of state are rare, i.e., a maximum of one per branch (Cunningham et al., 1998), which may be a weakness particularly when analyzing characters at higher taxonomic levels. In addition, the chance of a state change occurring is considered the same on every branch regardless of length, and MP does not provide probabilistic estimates of error or support for ancestral states, showing only the most parsimonious even if only marginally so. In contrast, probabilistic, model-based approaches (including maximum likelihood [ML] and Bayesian methods) can incorporate measures of uncertainty and/or probability in ancestral states and, where an appropriate model is selected, make use of branch length information (Cunningham et al.,

1998). However, these methods tend to be sensitive to taxon sampling and to the accuracy of branch lengths (Torices, 2010). Of these, ML techniques optimize ancestral states so as to maximize the probability of the observed states in the terminal taxa, given a single explicit model of evolution (for morphological data this is usually simple, such as a one-parameter Markov [Mk-1] model, in which the single parameter is the rate of change). ML analysis assumes a constant evolutionary rate across the tree, i.e., the likelihood of change along a branch is proportional to its length (Cunningham et al., 1998). For phylogenies based on molecular evidence, this may be problematic if the morphological events of interest are uncoupled from molecular change, such as when morphological change is concentrated during speciation events. The technique sometimes known as empirical Bayesian (EB) inference is considered by some authors to be superior to ML methods (e.g., Yang, 2006). Like ML, it uses an Mk-1 model of transition rates derived from the data, but unlike the former it subsequently derives posterior probability distributions for ancestral states. Fully (hierarchical) Bayesian (HB) techniques have the advantage that they can incorporate multiple alternative base phylogenies, by calculating relative probability for each possible character state at each node on a set of trees sampled from a distribution (usually generated during Bayesian inference of phylogeny). However, some studies have suggested that the advantages of optimization over multiple phylogenies are minimal (Hanson-Smith et al., 2010). Possible disadvantages of Bayesian methods include their dependence on the choice of prior probabilities, which may be somewhat arbitrary. Both ML and Bayesian methods are limited in applicability since they can at present be conducted only on fully resolved phylogenies and datasets without polymorphisms.

Although the starting tree we employed for comparisons of coding and optimization method (Jansen et al., 2007) was the most reliable and suitable phylogeny available for this study, it is not the only possible representation of relationships between angiosperm taxa. Recent studies have found a variety of patterns among some of the earliest branching lineages of the angiosperm tree, and even a thorough analysis of whole genome data, aimed specifically at resolving relationships among basal angiosperms, failed to reach resolution on this issue (Moore et al., 2007). Although the majority of plastid-based studies have agreed on the topology found by Jansen et al. (2007), since topology is crucially important to polarizing character states we take the conservative view of Soltis et al. (2008) that this should not be considered settled until comparisons have been made with nuclear sequence data.

Materials and Methods

Study taxa

Study taxa were determined by the starting phylogeny (Fig. 2), which was taken from the work of Jansen et al. (2007), and comprised 64 taxa in 59 genera (61 angiosperm species in 56 genera plus three gymnosperm outgroups). This phylogeny was selected from among those in the recent literature because it provided broad taxonomic coverage across a wide range of genetic loci, was fully resolved and well supported, featured branch lengths calculated with model-based methods, was based on the entire chloroplast genome (minimizing issues of rapid evolution at certain loci in certain taxa), and is highly congruent with other recent (including subsequent) phylogenetic estimates (e.g., Soltis et al., 2011). Pollen data were, if possible, taken from the same 64 species as sampled in the starting phylogeny. However, where palynological data were not available for these taxa, data were obtained from closely related species or genera (see Appendix 2 for a list of substituted taxa).

Pollen characters

Character state information was obtained from the literature, with taxon-specific palynological studies providing the primary reference where available. We also drew upon the broader studies of Sampson (2000) for magnoliids, Zavada (1983) and Furness and Rudall (2003) for monocots, Punt (1984; Umbelliferae) and Blackmore et al. (2009; Asteraceae), as well as Wodehouse (1935), Erdtman (1952), Walker and Doyle (1975), and Nowicke and Skvarla (1979; cf. Appendix 1). When possible, character state information was taken directly from original published images (light microscopy [LM], SEM, and TEM) and measurements rather than written descriptions, in order to achieve a more consistent description and terminology for character states across all taxa. All observations pertain to mature pollen grains. Differences in treatment and hydration state (for instance, of fresh material compared to that taken from herbarium specimens) can have a significant effect upon the nature of certain characters such as size and shape. Therefore, acetolyzed (Erdtman, 1960) grains were used wherever possible to ensure comparability of all characters both within this series and with the majority of palynological literature.



Figure 2. Angiosperm phylogeny redrawn from a maximum likelihood (ML) analysis of plastid genome data for 64 taxa, provided by Jansen et al. (2007), with branch lengths proportional to number of inferred nucleotide substitutions and taxon names as in the original article. This phylogeny served as the basis for most of the analyses presented here. Modified topologies used to test the effects of phylogenetic uncertainty in optimization of ancestral character states are described in the text. Clades represented by at least two taxa in the phylogeny are labeled if mentioned in the text.

As this is a preliminary study, character selection focused upon characters previously identified as variable and potentially informative at intra-ordinal level, including dispersal unit, polarity, symmetry, shape, size, aperture characters, exine structure and sculpture, and external features such as orbicules and viscin threads (Erdtman, 1952; Van Campo & Lugardon, 1973; Walker & Doyle, 1975; Skvarla et al., 1978; Nowicke & Skvarla, 1979; El-Ghazaly & Chaudhary, 1993; Schols et al., 2001; Merckx et al., 2008). For the purposes of data exploration, an inclusive approach was taken in which as many characters as possible were coded, including those that appeared to be somewhat subjective, poorly characterized, or potentially non-independent (note that non-independence of characters is only a concern for phylogenetic reconstruction, not for investigation of character evolution upon an independently derived phylogeny). For very complex features such as exine sculpture it remains difficult to design a perfect strategy for delimitation of characters and character states.

Palynology has several complex descriptive vocabularies, developed for the efficient portrayal of numerous features. The terminology used here follows that of Punt et al. (2007). One widespread shortcut used to avoid lengthy descriptions and complex terminology is the "pollen type."; Pollen types describe the entire nature of a pollen grain (size, shape, apertures, exine stratification, surface sculpture, etc.) in a single phrase such as type A, or Liguliflorae-type. While this approach is useful for the purpose of identifying isolated, dispersed pollen grains, as in fossil studies, to analyze pollen grain evolution, it is more appropriate to break down pollen types and compound terms into their most basic components, each conveying a character. For example, instead of the compound term ana-zona-sulcate we would describe aperture shape (zonate), orientation (latitudinal), structure (simple), and position (between the equator and distal pole). The palynological characters and character states investigated were as follows, organized in the sequence of Erdtman (1952; cf. Appendix 3 and Fig. 3).

Dispersal unit. The dispersal unit (Character 1) is the arrangement in which pollen grains are found at maturity and dispersal (Punt, 1962), also known as the pollen unit (Walker & Doyle, 1975). States range from monads (free grains; e.g., Fig. 3A) to permanent dyads (fused pairs), tetrads (fused groups of four, in various orientations; Fig. 3B), polyads (fused in defined multiples of four, to a maximum of 64; Fig. 3C), or pollinia (fused in larger, indeterminate numbers, to date only reported for Apocynaceae and Orchidaceae). In some taxa, notably the Cyperaceae, pollen grains are initially borne in tetrads but reduced by abortion to single grains, known as cryptotetrads or pseudomonads (Erdtman, 1952), soon after meiosis; we treat these structures as tetrads.

Polarity and symmetry. Polarity (Character 2) and symmetry (Character 3) are determined with reference to development in the tetrad: each pollen grain has a polar axis running outward from the center of the tetrad, and two poles where this axis meets the surface of the grain, a proximal pole at the center of the tetrad and a distal pole at the outer surface (Erdtman, 1952). Based on this, pollen grains may be apolar (Fig. 3A), isopolar (Fig. 3D), subisopolar, or heteropolar (Fig. 3E). In apolar grains, such as many polyaperturate grains, orientation and polarity are impossible to establish at the free microspore stage. In isopolar grains, the two hemispheres are identical, as in most tri-aperturate, spheroidal, eudicot grains. Subisopolar grains are not entirely symmetrical about the equatorial plane, e.g., due to the addition of viscin threads on one face of the grain. In this paper, we treat subisopolar grains as isopolar, since they are usually the same in fundamental structure. In heteropolar grains, the two polar hemispheres differ distinctly in shape or apertures (Erdtman, 1952).

When viewed from either pole, pollen grains typically have either two (bilateral symmetry; Fig. 3F) or more than two (radial symmetry; Fig. 3G) planes of symmetry running perpendicular to the equator. The former is typical of monocolpate grains, the latter of most tri-zono-aperturate grains (Erdtman, 1952).

Shape. The basic three-dimensional shapes of pollen grains (Character 4) are often categorized as either globose or boat-shaped. Other shapes, such as threadlike, are restricted to a few species with reduced exines. Boat-shaped pollen, found in many grains with a single, colpate, polar aperture, has a short polar axis and unequal equatorial axes (Walker & Doyle, 1975; Fig. 3F). Globose pollen, the most common type, is approximately spheroidal to ellipsoidal (e.g., Fig. 3A, D, E, G–P). These two broad states are included in our matrix in order to assess whether the character may be informative. However, since many grains do not precisely fit such categories, it has become more conventional to describe shape in terms of two outlines: in equatorial and in polar view. In equatorial view, grains may be defined by their shape class (Character 5; Erdtman, 1952), which is defined as the ratio of the lengths of their polar and

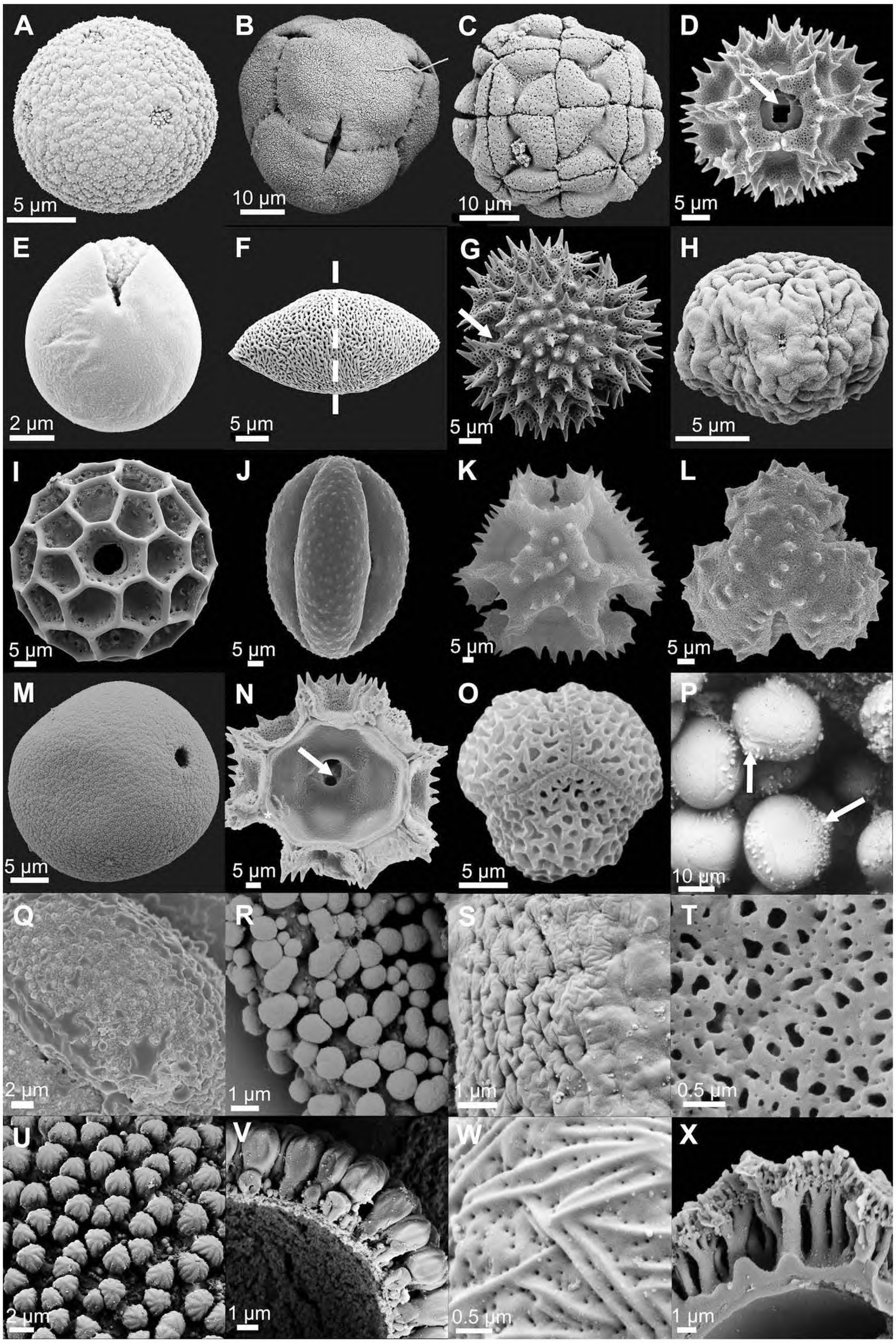


Figure 3. SEMs of pollen grains representative of some of the characters and character states used in this paper. For voucher details see Appendix 6. A–P. Whole grains or clusters of grains. —A. *Plantago psyllium* L. (Plantaginaceae), unknown view, illustrating dispersal unit (Character 1) as monads, pollen grains apolar (Character 2), basic shape (Character 4) globose, apertures (Character 8) many, aperture position (Character 9) global. —B. *Rhododendron wallichii* Hook. f. (Ericaceae), dispersal unit (Character 1) as tetrads. —C. *Acacia nilotica* (L.) Willd. ex Delile (Fabaceae), dispersal unit (Character 1) as polyads. —D. *Crepis napifera* (Franch.) Babc. (Asteraceae), equatorial view, pollen grains isopolar (Character 2), aperture membranes (Character 12;

equatorial axes (P/E). A P/E of less than 0.5 is defined as peroblate, 0.5 to 0.75 as oblate (Fig. 3H), 0.75 to 1.33 as subspheroidal (Fig. 3I), 1.33 to 2.0 as prolate (Fig. 3J), and greater than 2.0 as perprolate (Erdtman, 1952). The widespread subspheroidal class may further be divided into four categories: suboblate (P/E 0.75–0.88), oblate-spheroidal (P/E 0.88–1.0), prolate-spheroidal (P/E 1.0–1.14), and subprolate (P/E 1.14–1.33; Erdtman, 1952). In polar view (amb; note that in heteropolar grains this does not necessarily coincide with the equatorial outline), the outline (Character 6) may be described as circular (Fig. 3I), elliptical, polygonal (Fig. 3K), concave-polygonal (angular with concave sides), or lobate (curved with convex sides separated by indentations; Fig. 3L).

Size. We follow the convention of Erdtman (1952) and Walker and Doyle (1975) in measuring size (Character 7) as the length of the longest axis of each pollen grain. As a continuous character, size can only arbitrarily be divided into states; furthermore, because size may vary with preparation of the grain, it has been suggested that any states should be defined as separated at least by orders of magnitude (Walker & Doyle, 1975). We adopt the following standard classes (Erdtman, 1952; Walker & Doyle, 1975): very small (less than 10 μm), small (10–24 μm), medium (25–49 μm), large (50–99 μm), very large (100–199 μm), and gigantic (greater than 200 μm).

Apertures. Apertures, found in the majority of species, have been recognized as fundamental features of pollen grains (Wodehouse, 1928b; Erdtman, 1952; Walker & Doyle, 1975). They comprise modifications of the pollen wall such as openings, thinnings, or thickenings of the exine or intine for the purposes of interaction with the surrounding substrate, including the emergence of pollen tubes during germination. Erdtman (1952) considered seven aspects of aperture morphology, some of which may be interdependent: number, position, structure, shape, size, nature of the aperture membrane, and presence or absence of an operculum. In this paper, we consider all except aperture size. Note that while Erdtman (1952) described a series of terms indicating both position and shape of apertures, including sulcus (a furrow-shaped aperture situated at the distal pole), porus (a rounded aperture at the equator), and rugus (one of several, globally distributed furrows), for the present analysis we prefer to maintain the two characters (position and shape) as separate, for reasons described above.

The number (Character 8) and position (Character 9) of apertures is correlated with developmental factors during and after meiosis (Blackmore & Crane, 1988; Blackmore et al., 2007). In terms of number, in this study we use states zero, one (Fig. 3E, M), two, three (Fig. 3G, K, L), four to six, seven to 12, and more than 12 (Fig. 3A). In terms of position, apertures are defined as polar (proximal or distal; Fig. 3E), equatorial (Fig. 3G), or global (Fig. 3A).

←

arrowed) smooth, supratectal elements (Character 17) echinate. —E. *Acorus gramineus* Sol. ex Aiton (Acoraceae), equatorial view, pollen grains heteropolar (Character 2), apertures (Character 8) one, aperture position (Character 9) distal. —F. *Dioscorea nipponica* Makino (Dioscoreaceae), equatorial view, symmetry (Character 3) bilateral (dashed line marks single plane of symmetry), basic shape (Character 4) boat-shaped. —G. *Centrapalus pauciflorus* (Willd.) H. Rob. (Asteraceae), polar view, symmetry (Character 3) radial, apertures (Character 8) three, aperture position (Character 9) equatorial, aperture membranes (Character 12; arrowed) granulate, supratectal elements (Character 17) echinate. —H. *Ulmus glabra* Huds. (Ulmaceae), equatorial view, shape class (Character 3) oblate, tectum sculpture (Character 19) rugulate. —I. *Cabobanthus bullulatus* (S. Moore) H. Rob. (Asteraceae), equatorial view, shape class (Character 3) subspheroidal, ectoapertures (Character 11) porate, exine (Character 29) lophate. —J. *Nouelia insignis* Franch. (Asteraceae), equatorial view, shape class (Character 3) prolate, ectoapertures (Character 11) colpate. —K. *Tragopogon longifolius* Heldr. & Sartori (Asteraceae), polar view, outline (Character 6) polygonal, apertures (Character 8) three, supratectal elements (Character 17) echinate. —L. *Oldenburgia paradoxa* Less. (Asteraceae), polar view, outline (Character 6) more or less lobate, apertures (Character 8) three, tectum sculpture (Character 19) perforate. —M. *Poa bulbosa* L. (Poaceae), oblique view, apertures (Character 8) one, aperture structure (Character 10) simple, tectum sculpture (Character 19) areolate. —N. *Scorzonera hispanica* L. (Asteraceae), internal view of aperture, aperture structure (Character 10) compound, endoaperture (character not analyzed due to greater than 50% missing data; arrowed) lalongate, endexine (Character 23; asterisked) present. —O. *Illicium floridanum* J. Ellis (Illiciaceae), polar view, ectoapertures (Character 11) syncolpate (reproduced with kind permission of Elsevier from fig. 8 of Wang et al., 2009b). —P. *Aesculus hippocastanum* L. (Sapindaceae), multiple grains, operculum (Character 13; arrowed) present. Q–X. Details of exine sculpture and structure. —Q. *Dioscorea pyrenaica* Bubani & Bordere ex. Gren. (Dioscoreaceae), supratectal elements (Character 17) gemmate. —R. *Adenocaulon chilense* Less. (Asteraceae), detail of exine surface close to aperture, supratectal elements (Character 17) verrucate. —S. *Lathyrus pratensis* L. (Fabaceae), tectum sculpture (Character 19) fossulate. —T. *Cullumia rigida* DC. (Asteraceae), tectum sculpture (Character 19) reticulate. —U. *Croton argyratus* Blume (Euphorbiaceae), tectum sculpture (Character 19) *Croton*-patterned. —V. *Croton argyratus*, fractured exine in cross-section, tectum sculpture (Character 19; shown in cross-section) *Croton*-patterned. —W. *Dampiera stricta* (Sm.) R. Br. (Goodeniaceae), tectum sculpture (Character 19) striate. —X. *Alfredia cernua* (L.) Cass. (Asteraceae), fractured exine in cross section, infratectum structure (Character 20) columellate.

Furthermore, for polygonal grains, the positioning of the apertures relative to the apices of the grain may be a useful character: in angulaperturate grains the apertures lie at the apices; in planaperturate grains they lie on the faces between the apices.

Aperture structure (Character 10) may be simple (Fig. 3M), comprising a single-layered thinning (ectoaperture) in the outer exine, or compound (Fig. 3N), in which there is also a thinning or opening (endoaperture) in the inner exine. Occasionally, a mesoaperture is present between the ecto- and endoapertures; its presence or absence is treated here as a binary character.

In terms of shape, the ectoaperture (Character 11) may be more or less porate (rounded; Fig. 3I), colpate (furrowed; Fig. 3J), zonate (encircling the grain in a ring), spiraperturate (encircling the grain in a spiral pattern), or syncolpate (with elongated apertures fusing, usually at the poles; Fig. 3O). The endoaperture (not analyzed here due to high levels of missing data) may be circular, endocingulate (encircling the grain in a ring), lalongate (elliptical or rectangular with the longest axis parallel to the equator; Fig. 3N), or lolongate (longest axis perpendicular to the equator).

The thin membrane that usually covers an aperture (Character 12) may be smooth (psilate; Fig. 3D) or beset with granules (Fig. 3G). In certain taxa, such as many Poaceae, the aperture membranes are conspicuously thickened with an island of ectexinous material toward the center, known as an operculum (Character 13; Fig. 3P). The apertures may also each be surrounded by an annulus (Character 14), a conspicuous thickening of the exine, which may take several forms: aspidate (outer exine thickened and protruding outward around the aperture), costate (inner exine, endexine, or foot layer thickened and protruding inward into the cytoplasm of the grain), or vestibulate (annulus subtended by a cavity).

Exine structure and sculpture. Most pollen grains are surrounded by an acetolysis-resistant sporopollenin exine (exceptions include many Zingiberales, some aquatic angiosperms, and a few Lauraceae). The exine is usually differentiated (Character 15) into distinct layers, for which there are two alternative descriptive systems. Erdtman (1952) defined two layers based on morphological observations only: an outer sexine and an inner nexine, both of which may be further subdivided into numbered layers. However, when comparing pollen grains with differing numbers of layers, this frequently results in non-homologous layers being given the same number. For this reason, we adopt the less ambiguous system of Fægri (1956) and Fægri and Iversen (1989). These authors defined terms ectexine (or ektexine) and endexine based on the developmental origins and staining properties of the layers. The endexine is usually single-layered, whereas the ectexine is often multi-layered, comprising a tectum and infratectum (which together correspond to the sexine of Erdtman [1952]) and foot layer (which, together with the endexine, comprises Erdtman's nexine). On the surface of the tectum there may also be supratectal elements.

Working inward from the external surface of the pollen grain, we first characterize the nature of the supratectal elements, if present (Character 16). In terms of shape (Character 17), they may be pilate (approximately cylindrical, greater in height than diameter), echinate (pointed, broader at base than at tip; Fig. 3D, G, K), gemmate (approximately spheroidal; Fig. 3Q), or verrucate (rounded and flattened, greater in diameter than height; Fig. 3R). Striate (parallel, elongated across the surface of the tectum) and rugulate (irregular, elongated across the surface of the tectum) supratectal elements were not observed in the present study. Supratectal elements can also be categorized by size (Character 18), which is here divided into two states, diameter greater than 1 μm (corresponding to macro-elements, usually visible under LM) and diameter less than 1 μm (micro-elements, usually only visible under electron microscopy).

Beneath the supratectal elements is usually a sculptured tectum. In LM studies, the sculpturing patterns are often categorized as OL- or LO-pattern following Erdtman (1952). Seen under the electron microscope, tectum sculpture forms are found to be numerous, varied, and not necessarily discrete or mutually exclusive; furthermore, their interpretation depends, to some extent, on the nature of both the supratectal elements above and the infratectum below (Punt et al., 2007). Here, we categorize states as follows (Character 19): perforate (with small, well-spaced openings less than 1 μm in diameter; Fig. 3L), foveolate (with large, circular, well-spaced holes greater than 1 μm diameter), fossulate (with elongate, irregular grooves; Fig. 3S), reticulate (with polygonal openings separated by narrow muri; Fig. 3T), areolate (with raised polygonal areas separated by narrow grooves; Fig. 3M), *Croton* L.-patterned (comprising groups of raised polygons arranged around a central space; Fig. 3U, V), rugulate (with irregular, elongate elements greater than 1 μm long; Fig. 3H), striate (with elongate parallel elements and grooves or spaces between; Fig. 3W), striato-reticulate (with elongate parallel elements and cross-links between), and imperforate (without sculpture, sometimes re-

ferred to as a "complete tectum" sensu Erdtman [1952]). There also exists a unique tectum sculpture reported only for *Amborella trichopoda* Baill. (treated in more detail by Lu et al., 2014, this issue), which comprises small cupules constructed of coiled cylindrical strands (Sampson, 2000).

Beneath the tectum lies the infratectum (Character 20), which is limited to one of three forms in almost all seed plant species (Van Campo & Lugardon, 1973): alveolate (restricted to gymnosperms and extinct, non-angiosperm seed plants), columellate (restricted to angiosperms; Fig. 3X), and granulate (observed in some species of angiosperms, most notably Annonaceae, and independently in some gymnosperms such as Gnetales). The infratectum itself may in some taxa (e.g., many Asterales) be distinguished into a number of layers (Character 21).

Below the infratectum are the foot layer (Character 22) and endexine (Fig. 3N), if present (Character 23). The endexine may differ in nature (Character 24: compact, spongy, or lamellar at maturity) and extent (Character 25: continuous, discontinuous, or solely apertural). In some taxa (e.g., Asteraceae), one or more exine layers are separated by a cavity known as a cavea, coded here as present or absent (Character 26). Also in some taxa (most notably Asteraceae), the elements that make up the exine are themselves perforated with tiny holes known as internal foramina (Character 27). In other taxa, the exine is traversed by minute, radially orientated channels (microchannels; Character 28). The latter two characters are usually visible only under TEM, and their presence or absence is rarely reported in the literature. In certain taxa (including many Asteraceae such as Fig. 3I), the exine may be folded into protruding ridges (lophae) and depressions (lacunae; Character 29), forming a pattern described as lophate (Wodehouse, 1935; Punt et al., 2007). Pollen that is not fully lophate but bears supratectal elements in a pattern as if upon lophae is described as sublophate.

External structures. Two types of structures external to the pollen grain, possibly of tapetal origin, have been highlighted in previous studies. Viscin threads (Character 30) are acetolysis-resistant, sporopollenin strands arising from the surface of the exine, common in some Onagraceae and Ericaceae. Orbicules (Character 31) are granules of sporopollenin found on the supratectal surface, sometimes referred to as Ubisch bodies. Although frequently reported from the surface of the anther locules, usually in species with a secretory tapetum, we record these only when they are found on the surface of the mature pollen grains themselves.

COMPARISON OF METHODS FOR STUDYING TRAIT EVOLUTION

In this paper, we test three aspects of the methodology for estimating ancestral states of pollen morphological characters upon a phylogeny of higher taxa: coding strategy, optimization method, and base tree topology.

Coding strategy. Because this study is based on a phylogeny of higher-level (ordinal) relationships in angiosperms inferred from character states (molecular sequence data) for individual species, we tested three methods of coding higher taxa for ancestral state optimization, represented in three different matrices: the species exemplar method (using mostly single exemplars; the possibility to select multiple exemplars was not available as the terminal taxa were preselected); the comprehensive method (where possible coding at least six species from across the phylogenetic and morphological range of each order, and displaying all observed states, with taxa selected to represent orders based on the delimitations of 2009); and the democratic method (sensu Bininda-Emonds et al. [1998]; a matrix created from the comprehensive matrix but with polymorphic data points reduced to the most common state. When two states were found to be equally prevalent, the state was coded as unknown). We did not test the ancestral method because neither fossils, ontogenetic data, nor existing phylogenies were widely available. We also created a modified species exemplar matrix in which polymorphic data points were reduced to the unknown state (?).

Coding strategies were compared for ancestral state optimization using the MP algorithm implemented in Mesquite versions 2.6–2.72 (Maddison & Maddison, 2009), because this is the only method of analysis currently applicable to matrices containing polymorphic cells, as found in two of the coding strategies tested. For consistency with tests of methodology, they were tested on a single phylogeny (Jansen et al., 2007). Consistency (ci) and retention indices (ri), as well as ensemble indices (CI, RI) were calculated for the four matrices on this phylogeny.

Optimization method. We tested four methods for optimization of ancestral character states: MP, ML, EB, and HB. Maximum parsimony optimization was conducted using Mesquite v.2.6–2.72 (Maddison & Maddison, 2009), with unordered (Fitch) parsimony under the "trace character history" option, and MacClade v.4.06 (Maddison & Maddison, 2000), using the "trace all possible changes" option. Maximum likelihood optimization was also conducted in Mesquite, applying an Mk-1 model, with param-

eters estimated from the data and using the default survey interval (two optimizations at a coarseness of 1.0 and 10.0, respectively). Empirical and hierarchical Bayesian optimizations were carried out using BayesMultistate (Pagel et al., 2004) in BayesTraits (<http://www.evolution.rdg.ac.uk/BayesTraits.html>). Results were examined in Microsoft Excel. Since the three model-based optimization methods are at present applicable only to datasets without polymorphic data points, to enable comparison across all four methods all were tested on the only two matrices that comply with this requirement: the democratic matrix and the modified species exemplar matrix (polymorphic data removed). The comprehensive matrix contained so many polymorphic cells that their removal would result in an unreasonably high proportion of missing data. For consistency with tests of coding strategy, they were tested on a single phylogeny (Jansen et al., 2007).

Testing multiple priors (see Appendix 4) indicated that the most appropriate methodology for HB inference with our data was to implement the reversible jump hyper prior (rjhp) mechanism as recommended in BayesTraits. This was applied for all characters, starting with a run of 100,000 iterations, sampling every 100 generations and a burn-in of 1000 generations. Rjhp settings (prior distribution type, range for uniform seeding distribution, and rate deviation) were manipulated for each character to ensure that the distribution of transition values included the point estimates obtained from the EB analysis, and so as to obtain a mean acceptance value of 20%–40% (minimizing autocorrelation among successive states of the chain while still exploring parameter space thoroughly; Pagel & Meade, 2006). Tests suggested that sampling every 300 generations successfully avoided autocorrelation between adjacent reported results. Plots of posterior values suggested that a burn-in period of anything greater than 10,000 generations was ample. Thus, the final parameter settings for the HB analysis were to sample every 300 generations, with a burn-in period of 20,000 generations for a net total of 5,000,000 generations (i.e., 5,020,000 including the burn-in period).

At each node, the favored states (i.e., the most parsimonious, most likely or those with the greatest posterior probability) generated by the four methods were compared. Where MP optimization gave multiple most parsimonious states, these were all considered equally supported and, thus, the method to be congruent with other methods if any of the states agreed. Similarly, where no single state was reconstructed for a node under ML (usually due to missing data), or where all states were found to be equally probable under Bayesian analysis, all states were considered to be possible and, therefore, the optimization to be congruent with any state generated by the other methods of analysis.

Starting tree topology. Five alternative arrangements were tested, representing the most common and significant variations found in recent, comprehensive phylogenetic studies (a wider range of topologies will be investigated in the second paper in this series (see Lu et al., 2015), focusing on basal angiosperms): (1) *Amborella trichopoda* sister to all other angiosperms, with the Nymphaeales the next branching clade, Chloranthales sister to magnoliids, and monocots sister to eudicots—this topology was obtained, with strong support, by Jansen et al. (2007; Fig. 2); (2) *A. trichopoda* sister to Nymphaeales, together sister to the remainder of angiosperms, Chloranthales, magnoliids and eudicots forming a polytomy and together sister to monocots—this topology was obtained by Soltis et al. (2007); (3) *A. trichopoda* sister to Nymphaeales (sensu Soltis et al., 2007), Chloranthales sister to magnoliids, and monocots sister to eudicots (sensu alternative topology in Jansen et al., 2007); (4) *A. trichopoda* sister to all other angiosperms (sensu Jansen et al., 2007), Chloranthales, magnoliids and eudicots forming a polytomy and together sister to monocots (sensu Soltis et al., 2007); (5) relative positions of both *A. trichopoda* and the Nymphaeales, and Chloranthales, magnoliids, monocots, and eudicots, left unresolved.

Topologies were compared for ancestral state optimization using the MP algorithm implemented in Mesquite versions 2.6–2.72 (Maddison & Maddison, 2009). Model-based methods of analysis were not appropriate because not all phylogenies were associated with branch lengths. For consistency with tests of methodology, this was conducted using the modified exemplar species matrix (polymorphic data removed).

Results

POLLEN CHARACTERS

After initial documentation of 35 palynological characters, three were removed due to extensive (greater than 50%) missing data (viz. aperture position in polar view, mesoaperture presence/absence, and endoaperture shape) and one due to invariability (tectum presence/absence). In the resulting matrix coded according to the exemplar method, five characters were parsimony-uninformative (and entirely invariant in the democratic matrix)

but were retained for their potential usefulness in model-based analyses (viz. Character 15, exine differentiation; Character 26, cavea; Character 27, internal foramina; Character 29, exine folding; Character 30, viscin threads). The final matrices (Appendix 5) contained 31 characters (Appendix 3). To enable complete comparisons across coding methods, all 31 characters were retained in the comprehensive and democratic matrices, even when they were invariant in these matrices.

COMPARISON OF METHODS FOR STUDYING TRAIT EVOLUTION

Coding strategy. The species exemplar matrix contained 137 cells (7%) missing data and 111 cells (6%) inapplicable, a total of 248 cells (13%) treated as missing data by the analysis programs. Seventy-six cells (4%) were polymorphic; when these cells were treated as missing data to facilitate comparisons across methods, the level of missing data increased to 326 cells (16%). The comprehensive matrix contained 42 cells (2%) missing data and 28 cells (1%) inapplicable, a total of 70 cells (4%) treated as missing data by the analysis programs. Eight hundred and twenty cells (41%) were polymorphic. The democratic matrix contained 97 cells (5%) missing data and 30 cells (2%) inapplicable, a total of 127 cells (6%) treated as missing data by the analysis programs. By definition, this matrix contained no polymorphic cells (see Table 1; Appendix 5). Although the ci varied between matrices, ri and rescaled consistency indices (rc) for characters followed generally similar patterns across the matrices, with the same characters having high or low indices in all three matrices (Table 2). Ensemble indices differed considerably, with CI 0.27, RI 0.5, and RC (ensemble rescaled consistency index) 0.14 for the exemplar species matrix, CI 0.51, RI 0.76, and RC 0.39 for the comprehensive matrix, and CI 0.35, RI 0.72, and RC 0.25 for the democratic matrix.

When analyzed using MP, the differences in inferred ancestral character state optimization between the three coding strategies were often large, both in terms of the location and number of character state changes inferred (Table 1). In general, the species exemplar matrix invoked the greatest number of character state changes, followed by the democratic matrix. For example, Character 2 (pollen grain polarity; Fig. 4) shows a complex pattern of multiple state changes when coded using exemplar species and optimized using MP (Fig. 4A). However, when coded comprehensively for orders, only a single state change is inferred, from heteropolar to isopolar (Fig. 4B). The exact position of the change within the phylogeny is ambiguous, occurring either at the root of the eudicots, or between Ranunculales and the rest of the eudicots. With democratic coding (Fig. 4C), the results are intermediate between these two extremes, with a clear transition from heteropolar to isopolar grains at the base of the eudicots, but further switches to apolar and isopolar grains seen closer to the tips of the phylogeny.

Optimization method. The two matrices used for comparison of optimization methods differed significantly. Of the 31 characters examined, 16 (52%) displayed different numbers of character states in the matrix generated by the democratic method of coding compared to that using exemplar species (Table 3), with the democratic matrix generally having fewer states than the exemplar species matrix. For eight characters, the democratic matrix contained only a single unambiguous state. In general, the democratic method of coding appeared to produce more consistent results across different methods of ancestral character state optimization: for the majority of characters (23), the democratic matrix generated fewer total disparities between methods than the species exemplar matrix (Table 4). The democratic matrix produced a greater number of differences for only two characters (Character 2, polarity, and Character 8, aperture number), while the remaining six characters generated the same total differences between methods with both matrices. Furthermore, the democratic matrix produced fewer differences at nodes for all six possible bilateral method comparisons. Therefore, the following comparison of methods is based on the species exemplar coding strategy, in order to avoid underestimating possible discrepancies between methods.

Notable differences were observed for many characters between the four different methods using the species exemplar matrix (Tables 3, 4; Fig. 5). By considering for each node the most parsimonious (from MP analysis), most likely (from ML analysis), and most probable ancestral state (from EB and HB analyses), it is possible to determine and quantify the differences between inferred ancestral states across all methods (Table 4). It should be noted, however, that such a comparison is relatively crude, taking no account of the relative likelihood (or probability) of each ancestral state, which in some cases was only marginally greater than that for the next most likely state. The level of congruence between optimization methods differed among characters. For example, four characters showed identical patterns with all methods tested: Characters 3 (symmetry), 13 (operculum), 21 (number of infratectum layers), and 29 (exine folding). Nine characters showed differences at more than 15 nodes (25%): Characters 5 (shape class), 6

Table 1. Comparison of pollen morphological character matrices examined in this study, generated using three alternative methods of coding higher taxa. C, comprehensive coding; D, democratic coding; E, species exemplar coding.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total
Number of states																																
C	5	3	2	2	5	5	5	7	3	2	5	2	2	4	2	2	4	2	11	3	2	2	2	3	3	2	2	2	2	2	2	n/a
D	1	3	2	2	2	4	3	5	3	2	2	2	2	3	2	2	4	2	7	2	2	2	2	3	3	2	2	2	1	2	2	n/a
E	3	3	2	2	4	4	5	7	3	2	4	2	2	4	2	2	3	2	11	3	2	2	2	3	3	2	2	2	2	2	2	n/a
Missing data (% cells)																																
C	0	0	0	0	6.3	0	0	0	0	0	0	1.6	0	0	0	0	14	14	1.6	1.6	1.6	1.6	3.1	23	11	1.6	14	17	0	0	0	3.6
D	0	0	1.6	6.3	6.3	3.1	4.7	1.6	3.1	0	4.7	4.7	4.7	4.7	0	4.7	19	17	14	1.6	7.8	1.6	4.7	31	16	1.6	14	17	0	0	3.1	6.4
E	0	0	1.6	0	13	0	0	0	4.7	4.7	4.7	20	4.7	4.7	0	0	50	50	3.1	1.6	4.7	22	22	48	45	1.6	36	45	0	0	0	12.5
Polymorphic cells (%)																																
C	69	58	45	16	70	89	94	92	39	48	81	53	45	63	0	83	64	56	95	6.3	6.3	16	20	7.8	9.4	6.3	7.8	0	6.3	3.1	3.1	41.3
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0	1.6	0	6.3	7.8	7.8	20	13	1.6	0	4.7	4.7	1.6	3.1	0	4.7	3.1	1.6	27	1.6	0	0	3.1	0	0	0	0	0	0	0	6.3	3.8
Number of state changes inferred under MP criterion																																
C	0	2	2	3	2	5	1	2	2	1	0	4	0	2	1	2	3	2	5	2	0	1	0	7	2	0	0	2	0	0	0	53
D	0	6	5	5	4	11	8	5	5	5	4	4	1	6	1	10	8	4	9	1	0	2	0	6	2	0	0	2	0	0	0	114
E	3	12	5	5	8	13	18	15	11	7	13	9	4	13	1	15	9	5	24	2	1	3	4	8	4	1	1	2	1	1	2	220

Table 2. Consistency (ci), retention (ri), and rescaled consistency (rc) indices for pollen morphological characters examined in this study, for three alternative methods of coding higher taxa, calculated on the phylogeny provided by Jansen et al. (2007). Final columns show mean across all characters and ensemble indices (CI, RI, and RC) for each matrix. For the exemplar coded matrix, values given include polymorphic data points; values when polymorphic data points were treated as missing data were similar; ensemble indices were CI 0.27, RI 0.5, RC 0.14.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Mean	Ensemble indices
Consistency index (ci)																																	
C	0	0.5	0.5	0.33	0.5	0.2	1	1	0.5	1	0	0.25	0	0.5	1	0.5	0.67	0.5	0.6	0.5	0	1	0	0.29	1	0	0	0.5	0	0	0	0.41	0.51
D	0	0.33	0.2	0.2	0.25	0.27	0.25	0.6	0.4	0.2	0.25	0.25	1	0.33	1	0.1	0.38	0.25	0.67	1	0	0.5	0	0.33	1	0	0	0.5	0	0	0	0.33	0.35
E	0.67	0.17	0.2	0.2	0.25	0.23	0.22	0.4	0.18	0.14	0.15	0.11	0.25	0.23	1	0.07	0.22	0.2	0.38	1	1	0.33	0.25	0.25	0.5	1	1	0.5	1	1	0.5	0.44	0.27
Retention index (ri)																																	
C	0	0.92	0.88	0.33	0	0.33	1	1	0.94	1	0	0.25	0	0.86	0	0	0.5	0	0.67	0.67	0	1	0	0.67	0	0	0	0.86	0	0	0	0.38	0.76
D	0	0.84	0.69	0.2	0	0.47	0.25	0.92	0.86	0.79	0.7	0.75	1	0.64	0	0.68	0.58	0.5	0.82	1	0	0.75	0	0.71	0	0	0	0.86	0	0	0	0.45	0.72
E	0	0.63	0.63	0.43	0.25	0.5	0.13	0.67	0.59	0.7	0.39	0.47	−.67	0.47	0	0.5	0.42	0.2	0.38	1	1	0	0.25	0.25	0	0	0	0.67	0	0	0.67	0.38	0.50
Rescaled consistency index (rc)																																	
C	0	0.46	0.44	0.11	0	0.07	1	1	0.47	1	0	0.06	0	0.43	0	0	0.33	0	0.4	0.33	0	1	0	0.19	0	0	0	0.43	0	0	0	0.25	0.39
D	0	0.28	0.14	0.04	0	0.13	0.06	0.55	0.35	0.16	0.18	0.19	1	0.21	0	0.07	0.22	0.13	0.55	1	0	0.38	0	0.24	0	0	0	0.43	0	0	0	0.2	0.25
E	0	0.1	0.14	0.09	0.06	0.12	0.03	0.27	0.11	0.1	0.6	0.05	0.17	0.11	0	0.03	0.09	0.04	0.14	1	1	0	0.06	0.06	0	0	0	0.33	0	0	0.33	0.14	0.14

Abbreviations: C, comprehensive coding; D, democratic coding; E, exemplar species coding.

(outline in polar view), 7 (size; Fig. 5A), 11 (ectoaperture shape), 12 (aperture membrane ornamentation), 14 (annulus; Fig. 5B), 17 (supratectal element shape), 19 (tectum sculpture), and 24 (endexine type; cf. Table 4). The greatest differences (> 35% of nodes) were seen for outline, size, annulus, supratectal element shape, and tectum sculpture. As an example, pollen size (Character 7; Fig. 5A) differed in optimization between methods at many key, deep nodes in the phylogeny. For instance, Node 6, which links *Pinus* L. and the angiosperms, had an inferred ancestral state of small under EB, medium under MP and ML, and large under HB. However, at more derived nodes (for instance, the root of the asterids and within this group), the four methods tended to converge on similar optimizations. In contrast, annulus type (Character 14; Fig. 5B) generated unanimous agreement between the methods at many of the deepest nodes in the phylogeny, such as the roots of angiosperms, monocots, and eudicots, all inferred as lacking an annulus.

In general, MP and ML tended to produce the most congruent results (differing at only 2.2% of possible nodes across all characters; Table 4); however, this may in part be due simply to the high number of equivocal optimizations generated by the ML analysis, which were treated as congruent with all possible states. The greatest incongruence was seen between the EB and the MP and ML methods (differing in 14.0% and 14.2% of optimizations, respectively).

Starting tree topology. For the five different topologies tested, using the species exemplar matrix (with polymorphic data points treated as missing data) and characters optimized with MP, very few changes were apparent in terms of ancestral state. The only characters for which any differences at all were noted were Characters 2 (polarity), 3 (symmetry), 4 (basic shape), 6 (outline in polar view), 9 (aperture position), 16 (supratectal elements), 19 (tectum sculpture), and 25 (endexine extent). In none of these instances did the differences affect key nodes (i.e., those deep in the phylogeny), and the number of state changes inferred was the same on all trees for all characters. The most significant difference noted was in Character 19 (tectum sculpture; Fig. 6). In this case, the state inferred at the root of the Nymphaeales differed depending on topology: for trees where *Amborella* Baill. and Nymphaeales were positioned on successive branches (e.g., Fig. 6A), the most parsimonious state for Nymphaeales was inferred as reticulate; for trees in which *Amborella* and Nymphaeales were sister groups (e.g., Fig. 6B), the most parsimonious state for the root of Nymphaeales was ambiguous, either reticulate or *Amborella*-type. This

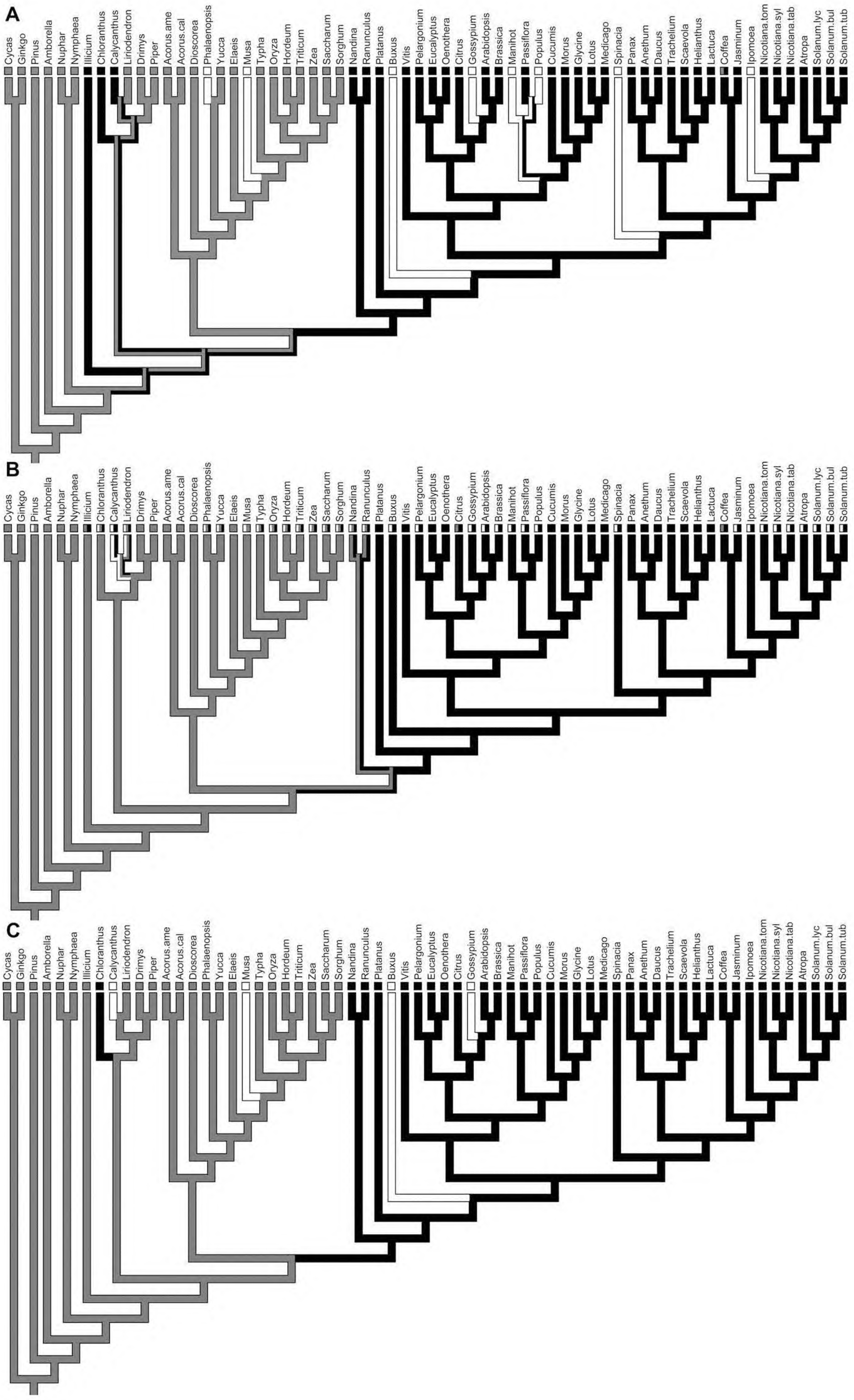


Figure 4. Comparing exemplar, comprehensive and democratic methods for coding higher taxa as terminals, using MP inference of ancestral states on angiosperm phylogeny, for the example of Character 2, pollen polarity: white, apolar; gray, heteropolar; black, isopolar/subisopolar. —A. Coding exemplar species as terminals (12 state changes inferred). —B. Coding taxonomic orders comprehensively as terminals (two state changes inferred). —C. Coding orders democratically as terminals (six state changes inferred). Branches displaying more than one shade indicate situations where the most parsimonious optimization is equivocal.

Table 3. Comparison of character statistics for matrices obtained using the democratic and exemplar (polymorphic cells treated as missing data—note that for this reason the number of states in the matrix may for some characters be fewer than the number of observed states) methods of coding higher taxa, calculated on the phylogeny provided by Jansen et al. (2007).

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total
Number of unambiguous (i.e., neither inapplicable, '-', nor unknown, '?') states																																
D	1	3	2	2	2	4	3	4	3	2	2	2	2	3	2	2	4	2	7	2	1	2	1	3	3	1	1	2	1	1	1	n/a
E	3	3	2	2	3	4	5	7	3	2	3	2	2	4	2	2	3	2	10	3	2	2	2	3	3	2	2	2	2	2	2	n/a
Number of state changes inferred under MP criterion (figures in **bold** indicate greater than 100% difference in number of steps [MP analysis] between species exemplar matrix and democratic matrix. Figures in *italics* indicate where difference may be due to total number of character states rather than differences in optimization)																																
D	***0***	6	5	5	*4*	11	*8*	***5***	**5**	5	***4***	**4**	**1**	*6*	1	10	*8*	4	***9***	*1*	***0***	2	***0***	6	2	***0***	***0***	2	***0***	***0***	***0***	114
E	***3***	12	5	5	*8*	13	*14*	***15***	**11**	7	***13***	**9**	**4**	*12*	1	15	*9*	5	***21***	*2*	***1***	3	***4***	8	4	***1***	***1***	2	***11***	***1***	***2***	122
Number of internal nodes differing in inferred state between D and E under the four optimization methods (figures in **bold** indicate where more than a quarter of the 63 internal nodes show disagreement in reconstruction between the two coding strategies)																																
EB	11	4	0	3	**25**	**16**	**23**	**25**	2	11	**23**	13	1	**21**	0	**17**	**29**	8	**29**	3	2	6	11	4	1	3	3	0	3	4	8	309
HB	2	2	0	1	**22**	**16**	**26**	3	1	**17**	11	5	1	12	0	**16**	**28**	3	**27**	2	2	0	9	7	2	0	0	0	0	0	2	217
ML	0	0	0	0	1	9	3	0	0	**19**	1	3	1	6	0	10	**18**	2	8	1	0	0	5	1	0	0	0	0	0	0	0	88
MP	0	0	0	2	1	4	5	0	0	**21**	5	3	2	4	0	9	9	3	14	1	2	0	1	0	0	0	0	0	0	0	2	88
Total nodes affected	11	5	0	3	**33**	**28**	**29**	**25**	2	**25**	**24**	**16**	2	**26**	0	**19**	**35**	12	**39**	3	2	6	11	10	3	3	3	0	3	4	8	390
Total differences	13	6	0	6	49	45	57	28	3	**68**	40	24	5	43	0	52	**84**	16	**78**	7	6	6	26	12	3	3	3	0	3	4	14	704

Abbreviations: D, democratic matrix; E, species exemplar matrix; EB, empirical Bayesian inference; HB, hierarchical Bayesian inference; ML, maximum likelihood; MP, maximum parsimony.

Table 4. Comparison of observed node differences between optimization methods for matrices obtained using the democratic and exemplar (polymorphic cells treated as missing data) methods of coding higher taxa, calculated on the phylogeny provided by Jansen et al. (2007). Figures in **bold** indicate where more than 25% of the 63 internal nodes showed disagreement in reconstruction between the two coding strategies.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total
Number of observed differences (democratic matrix)																																
MP-ML	0	0	0	0	0	0	0	0	0	5	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	8
MP-EB	0	9	0	8	14	19	18	17	8	5	3	4	0	15	6	3	7	0	5	0	0	0	0	11	2	0	0	0	0	0	0	153
MP-HB	0	0	0	10	16	19	0	1	1	5	0	3	0	1	0	3	3	0	3	0	0	0	0	6	3	0	0	0	0	0	0	74
ML-EB	0	9	0	11	12	22	17	17	6	2	5	3	0	14	6	3	7	0	6	0	0	0	0	10	2	0	0	0	0	0	0	152
ML-HB	0	0	0	12	14	22	1	1	1	4	0	2	0	1	0	4	3	0	3	0	0	0	0	2	0	0	0	0	0	0	0	70
EB-HB	0	9	0	2	2	2	19	16	7	2	5	1	0	15	6	3	8	1	6	0	0	0	0	10	2	0	0	0	0	0	0	116
Total nodes affected	0	9	0	15	**16**	**23**	**19**	17	8	7	5	4	0	15	6	6	10	1	8	0	0	0	0	**17**	5	0	0	0	0	0	0	191
Total differences	0	27	0	43	58	84	55	52	23	23	13	14	0	46	18	17	27	1	23	0	0	0	0	40	9	0	0	0	0	0	0	573
Number of observed differences (exemplar species matrix)																																
MP-ML	0	0	0	7	0	1	1	0	0	0	3	2	0	0	0	6	13	1	8	0	0	0	0	0	0	0	0	0	0	0	0	42
MP-EB	11	7	0	7	21	28	26	6	8	13	16	13	0	20	6	6	19	10	10	2	0	6	4	11	3	3	3	3	0	4	6	272
MP-HB	2	0	0	0	2	14	20	2	2	5	7	5	0	5	0	6	17	4	17	1	0	0	2	5	0	0	0	0	0	0	0	116
ML-EB	11	9	0	14	18	25	24	8	8	13	16	13	0	22	6	11	16	7	15	2	0	5	4	11	3	3	2	3	0	4	5	278
ML-HB	2	2	0	13	3	12	19	4	2	5	9	6	0	8	0	11	5	2	11	1	0	0	2	3	0	0	0	0	0	0	0	120
EB-HB	9	7	0	1	19	14	9	10	6	8	12	9	0	17	6	2	22	6	17	1	0	6	2	12	3	3	3	3	0	4	6	217
Total nodes affected	11	9	0	14	**22**	**18**	**27**	11	8	13	**20**	**16**	0	**24**	6	13	**25**	10	**25**	2	0	6	4	**18**	3	3	3	3	0	4	6	324
Total differences	35	25	0	48	63	94	99	30	26	44	62	48	0	75	18	42	92	30	78	7	0	17	14	42	9	9	8	9	0	12	17	1053

situation is partly an artifact of removing polymorphic states: both Nymphaeales taxa were coded as missing data for this character in this matrix, due to both having polymorphic tectum sculpture types, which led to the most parsimonious state inferred at the root of the Nymphaeales depending entirely upon the surrounding taxa.

OVERVIEW OF ANGIOSPERM POLLEN EVOLUTION

Since topology was found to make relatively little difference to ancestral character state optimization at the level of angiosperms for the broad-scale pollen characters investigated, the following discussion is based on a single topology (Jansen et al., 2007), which is associated with meaningful branch lengths derived from molecular genetic state changes, enabling it to be used with both model-based and model-free methods. Although the method of coding higher taxa was found significantly to impact the results of ancestral character state optimizations (see above), for the purposes of exploring the evolution of pollen characters the following discussion will be based on the matrix produced using the species exemplar method (polymorphic characters treated as missing data); this avoids overestimation of congruence between methods.

A number of characters showed structured patterns when optimized on the phylogeny of angiosperms and, therefore, have potential to provide diagnostic or synapomorphic characters and hypotheses of evolutionary processes. Unfortunately, these tend also to be the characters that differed in optimization depending on the coding strategy, optimization method, or topology used. In general, characters that were unambiguously optimized in all contingencies were either those for which little data were available (e.g., Character 28, microchannels), or those known to be variable only within a single group, such as the cavea (Character 26), internal foramina (Character 27), and lophate exine (Character 29) of certain Asteraceae.

Nonetheless, of the 31 characters tested, only eight (Characters 5, 17, 18, 21, 25 to 27, and 29) were relatively uninformative. These were either variable only within a very small group (e.g., Character 29, exine folding), lacking in data (e.g., Character 17, supratectal element shape), or highly homoplastic at the present hierarchical level (e.g., Character 5, shape class). The remaining 23 characters were of some interest from an evolutionary or taxonomic point of view, despite variability across optimization methods. Several examples of particular interest are discussed below (Figs. 7, 8).

Character 2, pollen grain polarity (Fig. 7A), showed some differences in the ancestral character states inferred depending upon coding strategy, few differences among analysis methods, and no difference with tree topology. Optimization indicated a single inferred transition from heteropolar (the plesiomorphic state for angiosperms) to isopolar grains, most likely occurring on the internal branch between the stem of the monocots and the root of the eudicots (although it should be noted that further transitions are inferred on terminal branches, not shown in the figure, such as to isopolar in *Illicium* L., *Chloranthus* Sw., and *Calycanthus* L., and to apolar in *Ipomoea* L. and *Musa* L., among others).

Character 3, pollen grain symmetry (Fig. 7B), showed an entirely congruent pattern across all analysis methods and topologies tested, although optimizations differed with coding strategy. This character is inferred to have undergone two transitions from bilateral (the plesiomorphic state) to radial symmetry, once within monocots, on the branch leading to Zingiberales (represented by *Musa*) and Poales, and once on the internal branch between the stem of the monocots and the root of the eudicots. Again, there are further transitions within terminal branches (to radial symmetry in *Illicium*, *Chloranthus*, and *Drimys* J. R. Forst. & G. Forst.). However, radial symmetry seems to be fixed within eudicots, with no apparent reversals to bilateral symmetry among the taxa studied.

Character 4, basic shape (Fig. 7C), differed in reconstruction with tree topology and optimization method to a small extent but not with coding strategy. This character shows a clear transition from boat-shaped to globose occurring on the branch leading to Zingiberales and Poales, and another transition occurring some time after the divergence of the Nymphaeales from the rest of the angiosperms. With MP and ML, this latter change of state is inferred to have occurred before the divergence of the branch leading to Austrobaileyales, but with both Bayesian methods it is inferred prior to the root of the eudicots. Depending on the method, further transitions are invoked, e.g., to globose in the magnoliids, or a reversal to boat-shaped in early-diverging monocots. In all cases, globose pollen appears fixed within eudicots, with no apparent reversals to boat-shaped pollen. Due to high levels of missing data in basally branching taxa (polymorphisms within taxa removed for comparison of optimization methods), the plesiomorphic state at the base of the angiosperms was not resolved by all methods.

Character 6, outline in polar view (Fig. 7D), shows a much more complex pattern and displayed

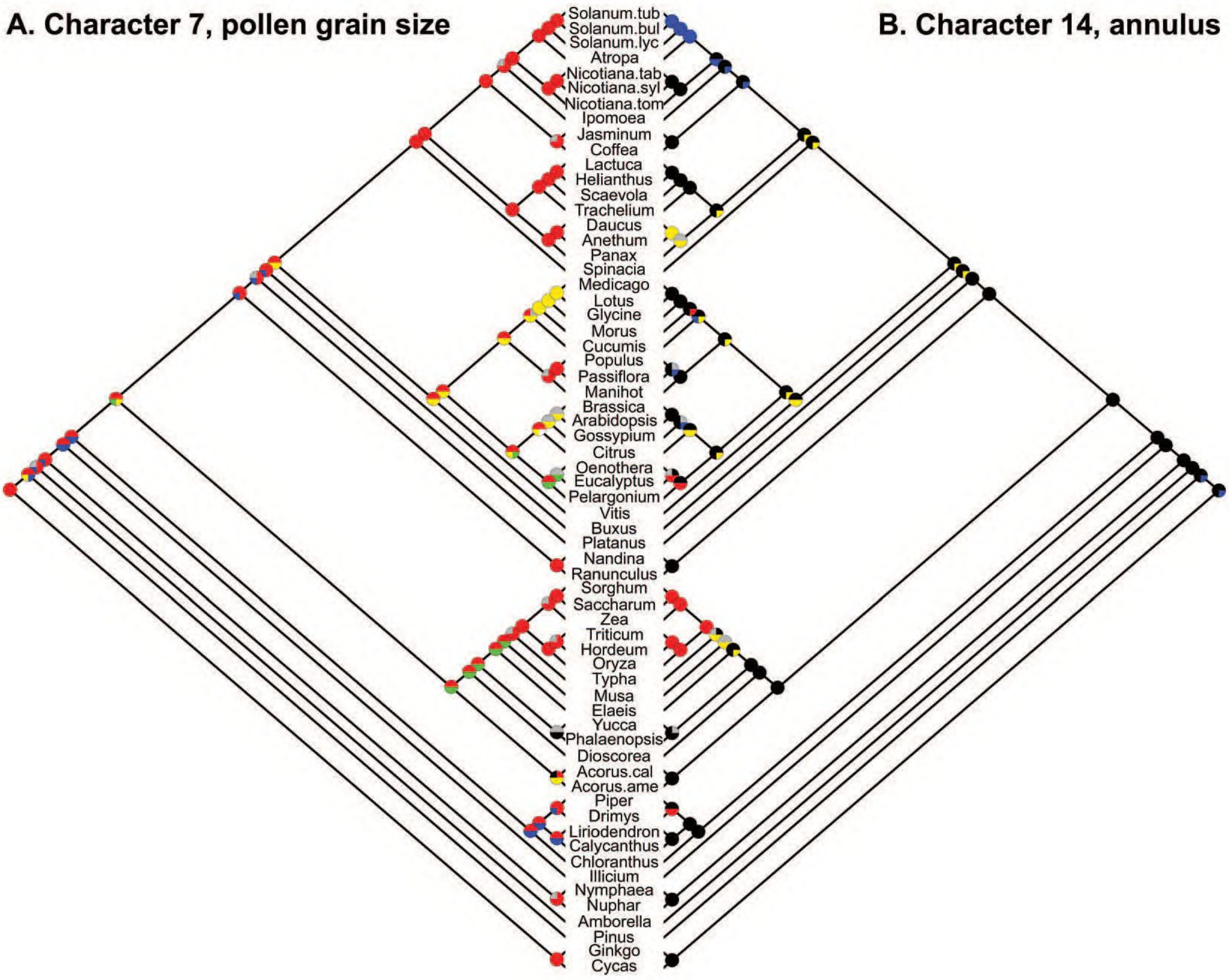


Figure 5. Comparing MP, ML, EB, and HB methods for inferring ancestral character states on angiosperm phylogeny, using species exemplar coding with polymorphic data points treated as missing data, for two example characters. Solid circles indicate congruence between all methods of optimization. Pie charts indicate incongruence: lower left segment, most parsimonious results; lower right segment, most likely result; upper right segment, most probable result of EB analysis; upper left segment, most probable result of HB analysis. —A. Character 7, pollen grain size: black, very small; yellow, small; red, medium; blue, large; green, very large. —B. Character 14, annulus: black, absent; yellow, costate; red, aspidate; blue, vestibulate. In both cases gray indicates an equivocal optimization.

differences with coding strategy, optimization method, and topology. The plesiomorphic state for angiosperms is resolved as oblate (although ambiguous in the ML optimization). In the two Bayesian optimizations, there is an inferred transition to circular outline before the branch leading to Austrobaileyales (represented by *Illicium*), in which case there is then a reversal to elliptic before the divergence of the monocots. All methods agree that there is a shift to circular grains within monocots and another before the root of the eudicots. However, in contrast to the previous characters, the situation within eudicots is highly complex and homoplastic, with little congruence between analysis methods on the exact placement of state transitions.

Character 8, aperture number (Fig. 8A), shows some discrepancies between optimization methods, particularly in inferring states at nodes toward the root of the tree. This character showed no differences in optimization between tree topologies but some differences between coding strategies. The plesiomorphic state for angiosperms (and several subsequent nodes) was inferred as mono-aperturate (MP and HB) or 4- to 6-aperturate (EB); the ML optimization was ambiguous at this point. All four methods were congruent in inferred state for the roots of monocots (mono-aperturate) and eudicots (tri-aperturate), with multiple transitions on terminal and subterminal branches to 4- to 6-, 7- to 12-, or poly-aperturate grains.

Character 9, aperture position (Fig. 8B), shows inferences differing slightly depending on coding strategy, optimization method, and tree topology. All methods inferred the plesiomorphic state for angiosperm apertures to be distal. Most methods (except EB) concurred on a likely transition to equatorial

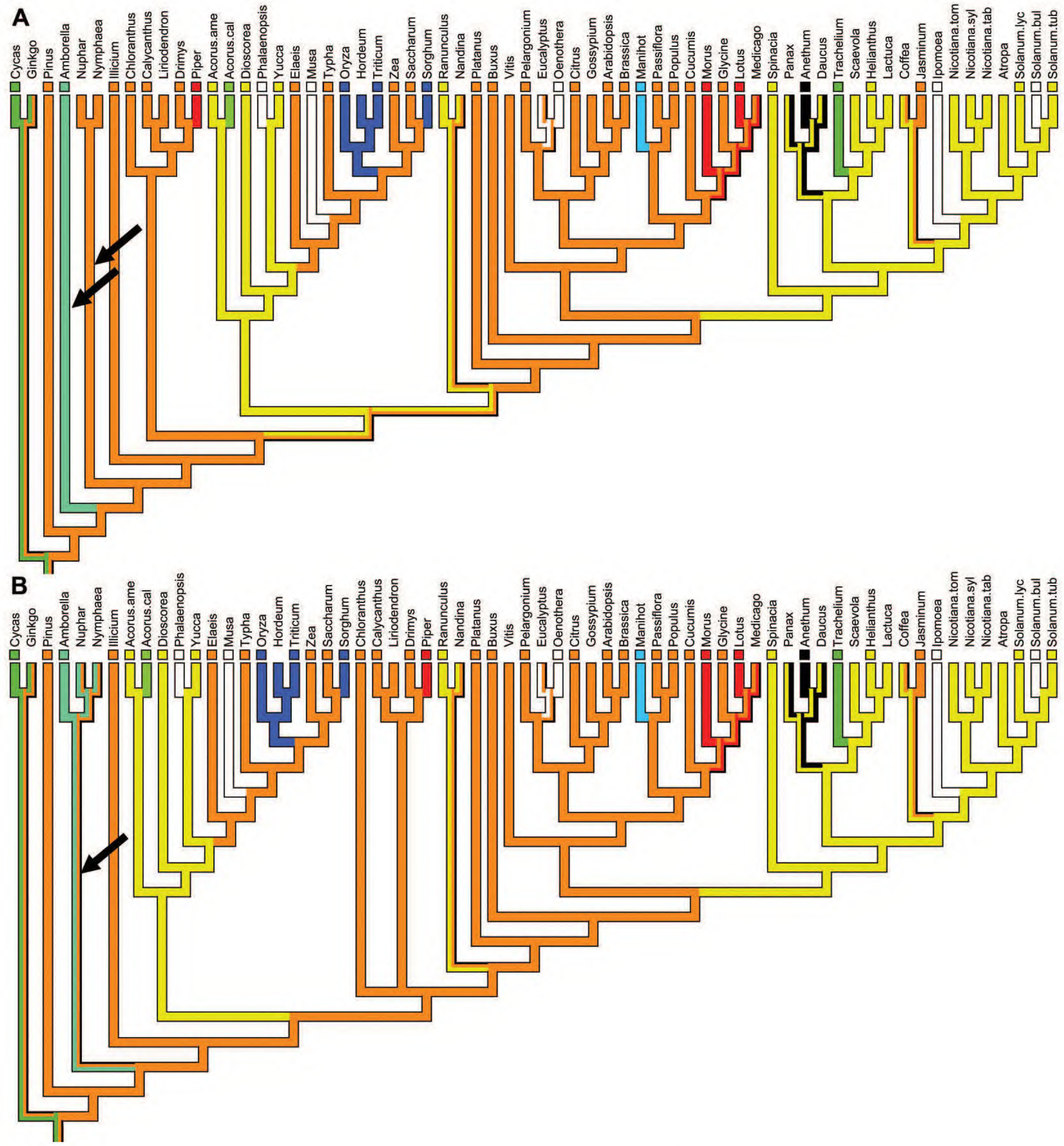


Figure 6. Comparing inferences of ancestral character state based on two different topologies for angiosperms, using the MP criterion and species exemplar coding with polymorphic data points treated as missing data, for the example of Character 19, tectum sculpture: white, imperforate tectum; dark blue, areolate; light blue, *Croton*-patterned; turquoise, *Amborella*-type; dark green, fossulate; light green, foveolate; yellow, perforate; orange, reticulate; red, rugulate; black, striate. Branches displaying more than one color indicate situations where most parsimonious optimization is equivocal. —A. Phylogeny as recovered by Jansen et al. (2007): *A. trichopoda* Baill. and Nymphaeales as successive sister groups to the remaining angiosperms. —B. Phylogeny as recovered by Soltis et al. (2007): *A. trichopoda* and Nymphaeales sister to one another. Arrows indicate groups where optimizations differ (see text for explanation).

apertures occurring on the branch leading to the root of eudicots. Multiple additional transitions to equatorial apertures are inferred on terminal taxa within the more basally branching angiosperms (such as *Illicium* and *Dioscorea* L.), and to global apertures within eudicots (e.g., *Gossypium* L., *Spinacia* L.). No transitions were inferred from distal to global apertures within the basally branching groups, nor any reversals to polar apertures within eudicots.

Optimization of Character 10, aperture structure (Fig. 8C), differed slightly between optimization methods, significantly with coding strategy, but not with tree topology. All methods concurred that the plesiomorphic state for angiosperms is simple

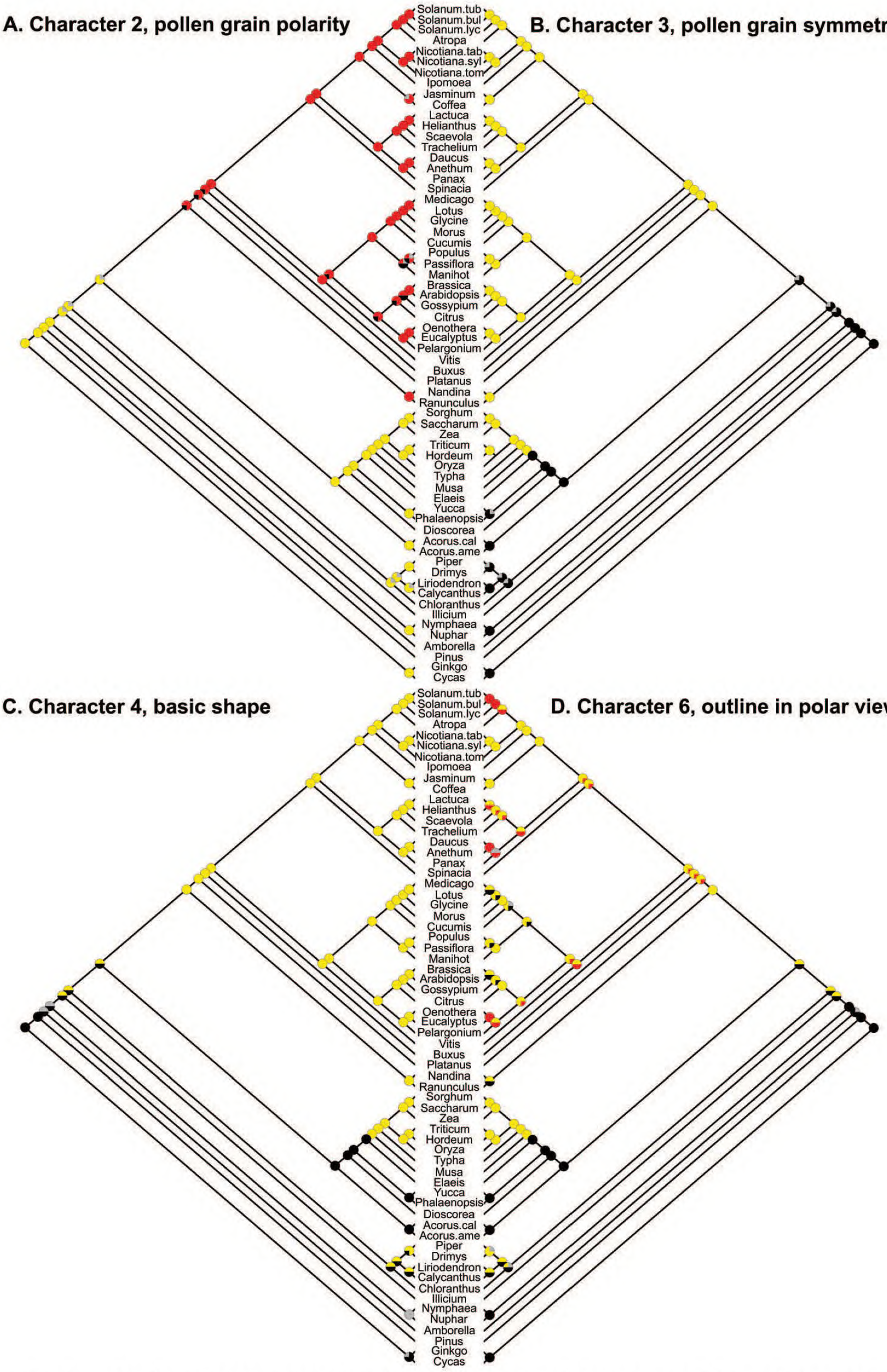


Figure 7. Examples of ancestral character states inferred on angiosperm phylogeny using all four analysis methods and the species exemplar coding method with polymorphic data points treated as missing data. Solid circles indicate congruence between all methods of optimization. Pie charts indicate incongruence: lower left segment, most parsimonious results; lower right segment, most likely result; upper right segment, most probable result of EB analysis; upper left segment, most probable result of HB analysis. —A. Character 2, pollen grain polarity: black, apolar; yellow, heteropolar; red, isopolar/subisopolar. —B. Character 3, pollen grain symmetry: black, bilateral; yellow, radial. —C. Character 4, basic shape: black, boat-shaped; yellow, globose. —D. Character 6, outline in polar view: black, elliptic; yellow, circular; red, polygonal; blue, lobate. In all cases gray indicates an equivocal optimization.

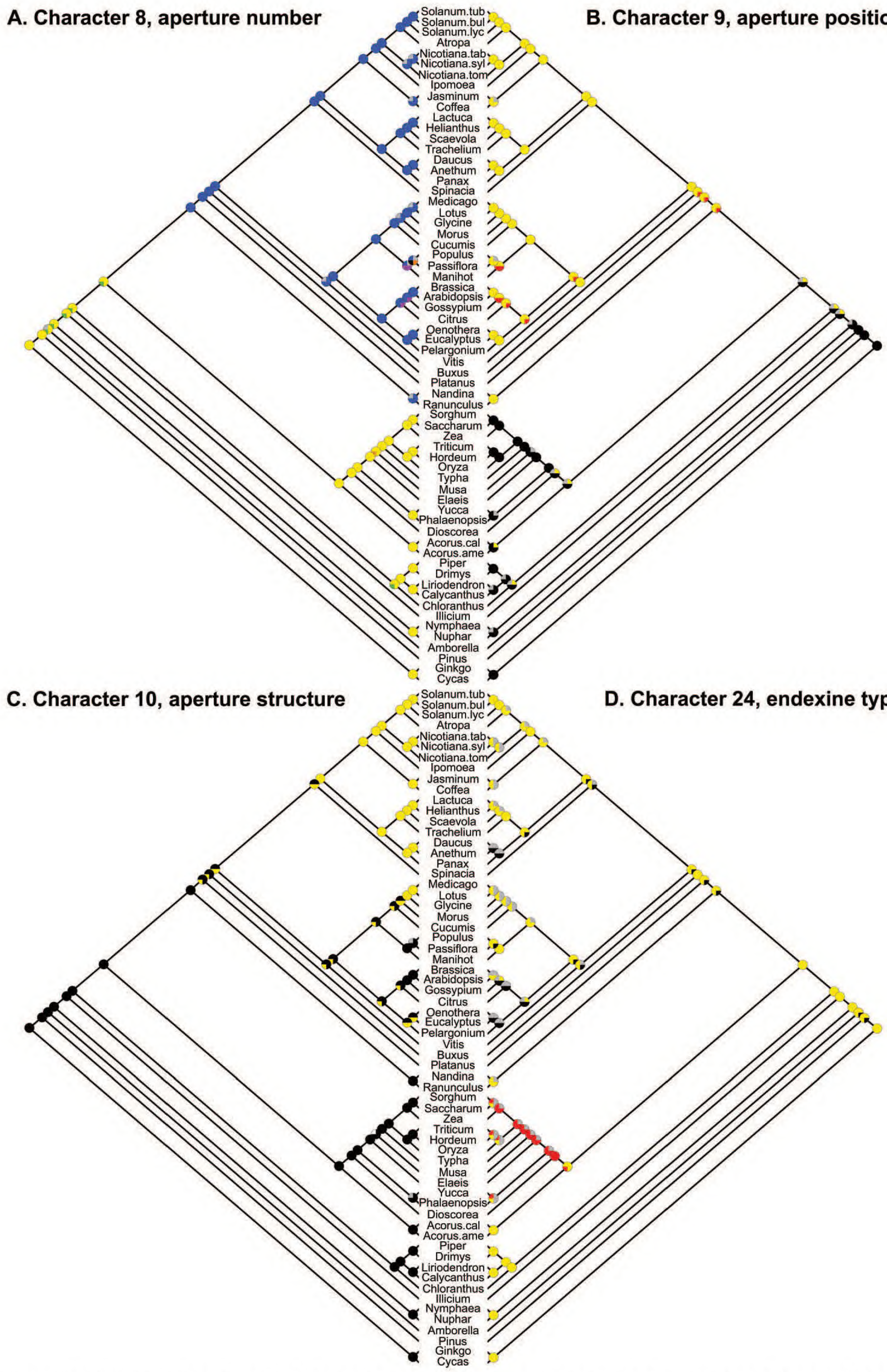


Figure 8. Further examples of ancestral character states inferred on angiosperm phylogeny using all four analysis methods and the species exemplar coding method with polymorphic data points treated as missing data. Solid circles indicate congruence between all methods of optimization. Pie charts indicate incongruence: lower left segment, most parsimonious results; lower right segment, most likely result; upper right segment, most probable result of EB analysis; upper left segment, most probable result of HB analysis. —A. Character 8, aperture number: black, zero; yellow, one; red, two; blue, three; green, four to six; orange, seven to 12; purple, many. —B. Character 9, aperture position: black, distal; yellow, equatorial; red, global. —C. Character 10, aperture structure: black, simple; yellow, compound. —D. Character 24, endexine type: black, compact; yellow, lamellar; red, granular/spongy. In all cases gray indicates an equivocal optimization.

apertures, a state that is retained until at least the separation of the basally branching eudicots (Ranunculales). However, the timing of the transition to compound apertures is more ambiguous. For example, under EB it occurs between the branch leading to Ranunculales and that leading to higher eudicots, under HB between Vitales (represented by *Vitis* L.) and higher eudicots, and under MP and ML only at the root of the asterids. Depending on the optimization method, further state changes are invoked on more terminal branches: either reversals to simple apertures (e.g., in *Oenothera* L. and *Spinacia*), or multiple origins of compound apertures (e.g., in Fabaceae and *Vitis*).

Our final example, Character 24, endexine type (Fig. 8D), was found to optimize in an entirely congruent manner across coding strategies and tree topologies, and to differ little between optimization methods. There are high levels of ambiguity in optimization due to the large amounts of missing data in this character; nonetheless, some interesting patterns are recorded. Most methods (with the exception of EB) concur that the plesiomorphic state for the angiosperm endexine comprises a lamellar structure at pollen grain maturity. Most methods also agree that there is a transition to granular/spongy endexine within monocots, above the branch leading to Acorales (with the exception of HB, which places this transition at the root of all monocots). The eudicots are inferred to be largely lamellar, with multiple transitions to a compact endexine structure.

When all characters are considered together in the context of the phylogeny, further patterns can be visualized. Figure 9 shows selected character state changes mapped using the MP (Fig. 9A) and HB (Fig. 9B) algorithms; ML and EB were not chosen for display due to their high rate of equivocal node reconstructions and large number of discrepancies with other methods, respectively. Maximum parsimony optimization inferred a total of 141 unambiguous character state changes (note that the vast majority of ambiguities resulted from missing terminal character states rather than multiple equally parsimonious optimizations). One hundred twenty of the inferred character state changes (85%) were autapomorphic at the family level or below and are excluded from Figure 9A for clarity. Hierarchical Bayesian optimization inferred a total of 283 unambiguous character state changes (with ambiguities due to missing data as well as to occasional occurrences of equal probabilities for each state at a node), 164 of which were (58%) autapomorphic at the family level or below, excluded from Figure 9B.

Figure 9A shows the 21 unambiguous character state changes that define interfamilial or higher-level groupings within angiosperms based on the MP optimization. Of the 45 nodes in the phylogeny (42 within angiosperms), 15 are associated with at least one inferred character state change, four are associated with two changes, and one with three changes (Fig. 9A). The node associated with the greatest number of inferred character state changes is that linking Zingiberales and Poales, which share transitions from bilateral to radial symmetry (Character 3), boat-shaped to globose pollen (Character 4), and elliptic to circular pollen in polar outline (Character 6), three characters which are not, of course, independent. The four nodes marked by two inferred character state changes are the root of the angiosperms itself, defined by a gain in supratectal elements (Character 16; albeit further modified many times within the phylogeny) and the transition from alveolate to columellate infratectum structure (Character 20); the root of the monocots, with transitions from globose to boat-shaped (Character 4) and circular to elliptic pollen (Character 6; both characters further reversed within the clade, in Zingiberales–Poales); the root of the commelinids (Arecales, Commelinales, Poales, and Zingiberales), characterized by transitions from a granular to smooth aperture membrane (Character 12) and perforate to reticulate tectum sculpture (Character 19; further modified several times on terminal branches within this clade); and the asterids + Caryophyllales (represented by *Spinacia*), defined by a further gain in supratectal elements (Character 16) and transition from reticulate to perforate tectum sculpture (Character 19). Key nodes characterized by a single inferred character state change under MP include that linking all angiosperms except *Amborella* and Nymphaeales (polar outline elliptic to circular, Character 6), that linking all angiosperms except the ANITA grade (Qiu et al., 1999; supratectal elements present to absent, Character 16), the root of the eudicots (aperture number one to three, Character 8), and the root of the asterids (aperture structure simple to compound, Character 10).

Figure 9B shows the 119 unambiguous character state changes that define interfamilial or higher-level groupings within angiosperms under HB optimization. Of the 45 nodes in the phylogeny, 36 are associated with at least one inferred character state change, five are associated with two changes, seven with three changes, five with four changes, two with five changes, and five with six changes, including *Phalaenopsis* Blume + *Yucca* L., which might represent the root of Asparagales, although sampling

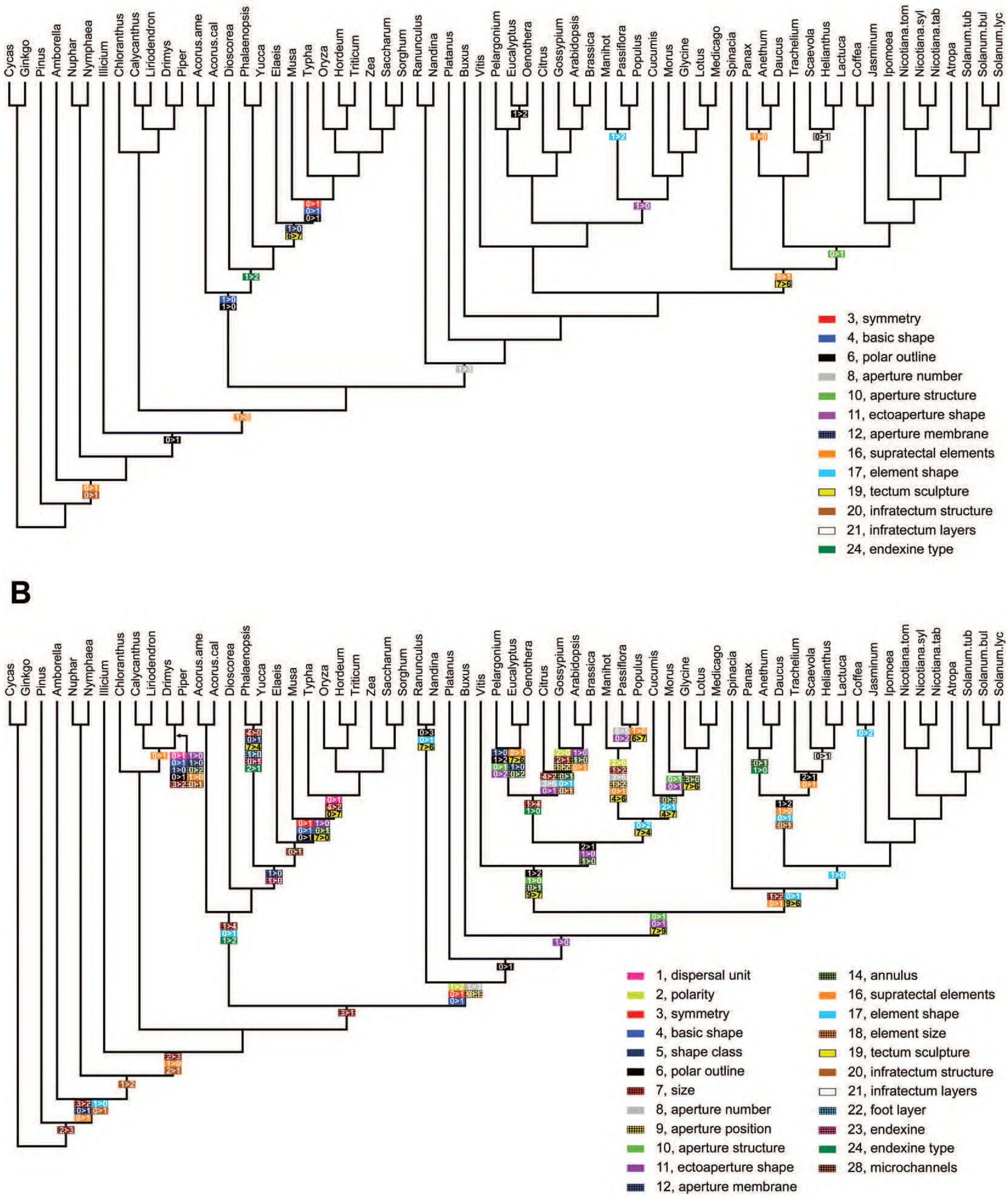


Figure 9. Summary evolution of pollen characters in angiosperms. Colors and patterns indicate characters (see legend for details), and numbers on bars indicate changes in state from left to right (for characters and states see Appendix 3). —A. The 21 unambiguous character state changes inferred using MP optimization of pollen characters at interfamilial level (changes autapomorphic for families or lower taxa removed), under species exemplar coding with polymorphic data points treated as missing data. —B. The 119 unambiguous character state changes inferred using HB analysis of pollen characters at interfamilial level (changes autapomorphic for families or lower taxa removed), under species exemplar coding with polymorphic data points treated as missing data. Changes are mapped assuming a single transition between differing states at adjacent nodes.

is minimal; and Zingiberales + Poales, characterized by porate, costate apertures (Characters 11, 14) and imperforate tectum sculpture (Character 19; although reversed at the next node), plus the three transitions observed in the MP analysis. One node is marked by eight character state changes (Geraniales [represented by *Pelargonium* L'Hér. ex Aiton] + Myrtales), and one with 10 changes (that linking Canellales [*Drimys*] and Piperales [*Piper* L.] within magnoliids). Two key nodes are characterized by five inferred character state changes: the root of the angiosperms, which in addition to the two transitions observed in the MP analysis is also marked by transitions from large- to medium-sized grains (Character 7), smooth to granulate aperture membranes (Character 12), and echinate to verrucate supratectal elements (Character 17)—all characters that change multiple times throughout the phylogeny; and the root of the eudicots, which, in addition to aperture number as observed in the MP analysis, is also distinguished by transitions from heteropolar to isopolar pollen (Character 2), bilateral to radial symmetry (Character 3), boat-shaped to globose (Character 4) grains, and distal to equatorial apertures (Character 9).

Discussion

In this first paper, we explore some of the available methods and issues to be considered when analyzing the evolution of morphological traits in a phylogenetic framework. Our analyses are based on a recent, comprehensive, model-based phylogeny for flowering plants (Jansen et al., 2007). Due to the patchy availability of genomic data, this phylogeny was heavily biased toward economically useful plants (such as Poaceae) and model taxa (such as *Arabidopsis* (DC.) Heynh. and *Nicotiana* L.). Nonetheless, it provided broad coverage of more than 50% of the orders and ordinal-level groups included in the APG III classification (APG III, 2009), and we believe this was a good starting point for study.

Comparison of methods for studying trait evolution

We tested three aspects of the methodology for inferring ancestral states upon a phylogenetic tree (coding strategy, optimization method, and tree topology), with 31 palynological characters. Certain characters, such as Characters 15 (exine differentiation), 28 (microchannels), and 29 (exine folding), were relatively robust to changes in the three parameters; these tended to be characters with low variability and high consistency indices, such as autapomorphies for one or a few taxa. Another set including Characters 6 (outline in polar view), 16 (supratectal elements), and 19 (tectum sculpture) showed high variability with all three parameters; these tended to be characters of high variability and homoplasy. However, many characters showed variability with some aspects of the methodology but not with others; these included Characters 2 (polarity), 7 (size), 12 (aperture membrane ornamentation), and 17 (supratectal element shape).

Coding strategy. The definition and coding of terminal taxa had a considerable impact upon ancestral character state optimization, due to its large resultant effect on the numbers of missing and polymorphic data points. Of the three coding strategies tested (species exemplar, comprehensive, and democratic), the greatest number of polymorphic data points was generated, as would be expected, by the comprehensive method, and the fewest by the democratic method (zero polymorphic data points as only one state was selected for each character in each taxon; cf. Table 1). The greatest amount of missing data occurred in the democratic matrix (due to removal of data points where states were of equal prevalence), and the lowest in the species exemplar matrix.

In general, the species exemplar matrix produced character state distributions with many more inferred changes of state than the other methods (Table 1; Fig. 4), while the comprehensive matrix resulted in the fewest inferred character state changes. This is clearly exemplified in Figure 4, for Character 2 (polarity), for which the species exemplar matrix generated 12 inferred changes of state, the democratic matrix six, and the comprehensive matrix two. This may be explained by the fact that the higher levels of polymorphism within terminal taxa in the comprehensive matrix are resolved, under MP, as changes within these terminal taxa, resulting in fewer character state changes on internal branches of the phylogeny (note that, for this reason, not all states shown in the matrices are always apparent in the figures; those that are autapomorphic for or within terminal taxa are not shown). By contrast, with the species exemplar coding strategy, any variability in the matrix occurs between terminal taxa, rather than within them, and thus is resolved as character state changes at internal nodes, increasing the number of inferred changes seen.

Characters that differed greatly when optimized based on different coding strategies tended to be major structural characters such as Characters 2 (polarity), 3 (symmetry), 10 (aperture structure), and 20 (infratectum structure). Aperture characters seemed to be particularly strongly affected, including Characters 10 (aperture structure), 11 (ectoaperture

shape), 13 (operculum), and 14 (annulus; cf. Table 1). A possible reason for this may be that aperture characters tend to be both highly informative at higher taxonomic levels and also variable at lower levels (i.e., within terminal taxa) resulting in differences in polymorphism levels with different coding strategies. We suggest that discrepancies due to coding strategy may be minimized in future studies with the use of probabilistic optimization methods, such as ML and Bayesian methods, which can accommodate proportional likelihoods at each node, and by increased taxon sampling, resulting in more accurate estimation of plesiomorphic states for higher-level taxa.

Optimization method. This study explores four methods (MP, ML, EB, and HB) of estimating character states for ancestral nodes and thereby inferring potentially diagnostic character states and evolutionary patterns. Previous studies have attempted empirically to compare the various techniques for ancestral state optimization in isolation, with mixed results. For example, Vanderpoorten and Goffinet (2006) found little difference between MP, ML, and Bayesian methods for morphological characters in the moss genus *Brachytheciastrum* Ignatov & Huttunen (Brachytheciaceae). In contrast, Ekman et al. (2008) found strong differences in the timing of the origin of the ascus in the Lecanorales (lichenized Ascomycota Caval.-Sm.), depending on the method used. Xiang and Thomas (2008) found differences between methods for highly homoplastic characters, but greater congruence for those with less homoplasy, when studying fruit and inflorescence characters in the Cornaceae. Wiens et al. (2007) found sometimes strongly supported and misleading differences between methods in a study of life history evolution in frogs.

Following an exploration of the impact of method under two coding strategies, we discuss the four methods of optimization in detail using only the species exemplar coding strategy. Our study found significant differences in ancestral state optimization between methods for most characters. Twenty-seven out of 31 characters differed to some extent, and 10 characters differed at more than 25% of internal nodes (Table 4). In general, the results of MP and ML optimizations were similar, with the results of the EB analysis differing most from the other three (Table 4). The MP method is perhaps most notable for the large number of ambiguous ancestral nodes recovered (Figs. 7, 8). The HB method produced the fewest ambiguous ancestral states, but it should be noted that states may be selected using this method, even if they have only a marginally greater inferred probability than alternative states (e.g., 51% compared to 49% for a binary character).

Figure 9B shows that the HB analysis resulted in many more character state changes that define groups (synapomorphies) than, e.g., MP (Fig. 9A); 42% of all character state changes inferred under HB define groups, compared to 15% under MP. This suggests that the HB method concentrates character state changes toward the base of the tree rather than on terminal branches (accelerates change), compared to MP. The EB method also tended to accelerate change compared to other methods (see, e.g., Fig. 8A, Character 8, in which aperture number increases at a much deeper node than with other methods).

The observed differences in ancestral character states reconstructed between optimization methods might be due to several factors, including optimality criteria, evolutionary models, and dependence (or independence) on branch lengths (in future studies using more than one base topology, this may also have an effect since only Bayesian methods can optimize character across more than one phylogeny at a time). Branch lengths seemed not to have a great effect: the number of character state changes inferred using ML and Bayesian methods was no greater on longer branches compared to MP. Most likely, the differences observed are due to the applied models of character state change, from the simple parsimony model, through the Mk-1 model used in ML and EB, to the complex multi-parameter continuous-time Markov models (Pagel & Meade, 2006) generated by HB. This raises the issue of whether the most complex models incorporating prior probabilities are based upon realistic assumptions, something of which some researchers are skeptical (e.g., Yang, 2006). Our tests applying a variety of priors (Appendix 4) indicated that, apart from the uniform, all priors produced generally similar results and, therefore, probably did not have a strong negative impact on any inferences of character evolution.

Figures 5, 7, and 8 demonstrate that in some cases the observed differences between methods would have a significant impact on inferences of character evolution or taxon diagnosis. For instance, Character 7 (size; Fig. 5A) was inferred under MP and ML to have evolved such that the ancestral condition toward the base of the tree was medium-sized pollen grains, while with Bayesian techniques many relatively basal nodes had large pollen size. For this character, the plesiomorphic states for angiosperms, monocots, and eudicots all differed between analysis methods (Fig. 5A).

Starting tree topology. For the phylogenies and characters explored here, tree topology had little

effect upon inferences of trait evolution using MP. Model-based optimization methods could not be used because branch lengths were unavailable for the multiple topologies, but we consider that the effect of topology would be similar regardless of the method used. Only eight characters (26%) showed slight differences in the manner in which their ancestral states were distributed (e.g., Fig. 6). In none of these instances did the differences affect key nodes (i.e., those toward the base of the tree), and the number of state changes inferred was the same on all trees for all characters. This is reassuring from the point of view of the analyses presented in this and future papers, because it indicates that, even where phylogenetic estimates may be uncertain, if the uncertainties involve only a few nodes and these are not within the regions of the phylogeny where character state changes are inferred to have taken place, there will be little negative impact upon inference of ancestral character states. Furthermore, where there are significant controversies in phylogeny estimation, and these do coincide with the likely timing of character state changes, multiple phylogenies with branch lengths in combination with probabilistic optimization methods can be used to explore all possibilities of trait evolution.

Since minimal differences were observed in the study presented here, the discussions that follow are based upon the single topology, from Jansen et al. (2007), for which branch lengths were available, making it possible to use both MP and model-based methods of optimization. We also focus on the exemplar species coding method, with polymorphic data points treated as missing data.

OVERVIEW OF ANGIOSPERM POLLEN EVOLUTION

Twenty-one (68%) of the characters examined showed relatively structured patterns when optimized on the phylogeny of angiosperms and, therefore, may have potential to provide diagnostic or synapomorphic character states, or to indicate key evolutionary transitions in pollen morphology. These included Characters 2 (polarity), 3 (symmetry), 4 (basic shape), 6 (outline in polar view), 8 (aperture number), 9 (aperture position), 10 (aperture structure), and 24 (endexine type; Figs. 7, 8). It should be noted that the many characters found to be highly homoplastic in our study (e.g., exine sculpture characters, size, shape in polar view) may also prove to be informative and diagnostic at lower levels with more detailed sampling.

Several characters show congruent patterns of inferred ancestral state distribution, with state changes located on the same branches of the phylogenetic tree. For instance, Characters 2 (polarity: apolar to heteropolar, with the exception of MP otimization; Fig. 7A), 3 (symmetry: bilateral to radial; Fig. 7B), 4 (basic shape: boat-shaped to globose, with the exceptions of MP and ML; Fig. 7C), 6 (outline in polar view: elliptic to circular, except MP and ML; Fig. 7D), 8 (aperture number: one to three, except MP; Fig. 8A), and 9 (aperture position: distal to equatorial, except ML and EB; Fig. 8B) all imply a change in state most likely on the branch subtending the root of the eudicots. These six character state changes that may be coincident with the origin of the eudicots are not independent but instead together form a switch in syndrome, from mono-aperturate (and, therefore, interpreted as heteropolar) to tri-aperturate grains in which the apertures are situated at the equator, rendering the pollen radially symmetrical, isopolar, and usually globose. A similar change in syndrome is suggested within monocots, where Poales and Zingiberales (represented by *Musa*) are linked by inferred changes from bilateral to radial symmetry (Character 3; Fig. 7B), boat-shaped to globose (Character 4; Fig. 7C) and elliptic to circular grains (Character 6; Fig. 7D), consistent wth a switch from boat-shaped grains with a single furrow to spheroidal grains with a single pore.

Key characters showing diagnostic changes at additional points on the phylogeny include aperture structure (Character 10) and endexine type (Character 24). For aperture structure (Fig. 8C), we can confidently infer a switch from simple to compound apertures within eudicots; however, the different optimization methods provide different conclusions on where this transition was most likely to have occurred: EB suggests it may have been as early as below the separation of the Proteales lineage from the remaining eudicots; HB suggests it occurred below the separation of the two large lineages corresponding approximately to rosids and asterids (with the caveat of limited taxon sampling); and MP and ML suggest that the main transition to compound apertures occurred just prior to the root of the asterids (with multiple additional occurrences in rosids). These differing interpretations provide a fruitful area for study because, while the adaptive function of compound apertures remains unclear, all possible optimizations suggest it could be linked to the evolution of highly diverse groups within angiosperms. The ambiguous optimization of this character may be partly dependent upon taxon sampling: e.g., the presence of known compound apertures within basally branching eudicot clades such as Buxales and Vitales implies that a single origin of compound apertures in the ancestor of all eudicots might be

most likely and would be in agreement with the interpretation of Blackmore and Crane (1998). This scenario adds yet another palynological synapomorphy for the eudicots. For endexine type (Character 24; Fig. 8D), there appear to be some clear patterns, including lamellar endexine in the more basally branching groups and a unique transition to granular or spongy endexine within monocots. In general, however, we are wary of drawing firm conclusions for characters such as this, with large proportions of missing, ambiguous, or polymorphic data, which may lead to artifactual results, such as the apparent occurrence of compact endexine at two of the deepest nodes when using EB analysis (Fig. 8D).

Aperture position (Fig. 8B) is an exceptionally interesting character because, despite the ambiguity and lability seen at certain nodes, some transitions were never observed to occur (within the sampling constraints of this study). For example, there are no occurrences within the outgroups, monocots, or basal angiosperms (either at terminals or internal nodes) of globally distributed apertures, and within the eudicots there are no observed reversals to distal apertures. This suggests that aperture position is a fundamental character, consistent with the fact that it is determined very early in pollen grain development by the nature of the meiotic tetrad.

In general, the palynological characters found to change state at key points in angiosperm evolution (particularly the origins of angiosperms and eudicots) tend to be those highlighted by previous authors (Wodehouse, 1935; Erdtman, 1952; Walker & Doyle, 1975; Nowicke & Skvarla, 1979). These include basic developmental characters such as polarity and symmetry, apertural characters, and the fundamental structure of the exine as granulate, alveolate, or columellate (Van Campo & Lugardon, 1973). However, additional characters also proved to show unexpected patterns. For example, in terms of shape, it was the simpler distinction between boat-shaped and rounded grains rather than quantitative shape classes (Fægri & Iversen, 1989), which was found best to distinguish groups.

Our results suggest that plesiomorphic pollen morphological states for the tree as a whole (i.e., in the common ancestor of the angiosperms and gymnosperms sampled) include monad-dispersed, heteropolar, bilaterally symmetrical, subspheroidal, medium-sized grains that are elliptic in polar view, with single, distal, simple, colpate apertures lacking an operculum or annulus. An exine is present, lacking supratectal elements, with a single-layered, alveolate infratectum; a foot layer and endexine are also present, the latter lamellated and continuous; and microchannels, viscin threads, and orbicules are absent. For a few characters the plesiomorphic state differed between optimization methods; e.g., MP recovered a plesiomorphic state of globose pollen, while HB suggested boat-shaped; HB implied that the plesiomorphic state for aperture membranes was smooth, while MP recovered it as granulate.

The highly variable pollen morphology seen in the most basally branching taxon of the ingroup, *Amborella* (polymorphic in terms of characters such as basic shape, outline, size, aperture number, ectoaperture shape, aperture membrane ornamentation, and endexine presence or absence), rendered recovery of plesiomorphic states for the angiosperms difficult, especially with conservative methods such as ML. Nevertheless, inferred plesiomorphic states for angiosperms themselves were found to include monad-dispersed, heteropolar, bilaterally symmetrical, subspheroidal, medium-sized grains that are elliptic in polar view, with single, distal, simple, colpate apertures having granulate membranes but lacking an operculum or annulus. An exine is present, with a reticulate tectum bearing echinate supratectal elements and a single-layered, columellate infratectum; a foot layer and endexine are also present, the latter lamellated and continuous; and microchannels, viscin threads, and orbicules are absent. Once again, MP recovered a plesiomorphic state of globose pollen, while HB suggested boat-shaped; MP implied that the plesiomorphic state for supratectal elements was echinate, while HB suggested verrucate.

From this we can see that angiosperms are distinguished from other seed plants by several synapomorphic pollen characters (Fig. 9), the least ambiguous being the presence of supratectal elements and columellate infratectum structure. Most of our analyses (except EB) confirm the discovery of Van Campo and Lugardon (1973) that columellate pollen defines and diagnoses the angiosperms. It is possible that the evolution of columellate structure, which incorporates a degree more complexity and flexibility than alveolate and granulate structures, is a key factor underlying the potential for the complex, multilayered exine structures seen in many groups of angiosperms (such as Asteraceae; Blackmore et al., 2009). This may in turn facilitate pollinator specificity, harmomegathy (the ability of pollen grains to change shape and size depending on hydration conditions), and other adaptive traits. It is important to note that the limited sample of taxa investigated here includes few angiosperms with granulate exine structure (key missing taxa include Annonaceae; Walker, 1971; Le Thomas & Lugardon, 1976), and

thus generates a simpler picture than might be revealed by fuller sampling. A deeper analysis of this particular character in a phylogenetic context is provided by Doyle (2009). None of the potential synapomorphies for angiosperms are ubiquitous; all display some reversals or further changes at lower hierarchical levels but nonetheless indicate a change in pollen type coincident with their origin.

Within angiosperms, there are several key points at which palynological characters may provide diagnoses or provoke evolutionary hypotheses (Fig. 9). The branch below the separation of Austrobaileyales (represented by *Illicium*) from the rest of eudicots is characterized by a switch from elliptic to circular grains (MP analysis; this state change is reversed at other levels in the phylogeny) or, under HB analysis, by two highly homoplastic character state changes (size large to medium, supratectal elements present to absent) and one very consistent character: infratectum structure granulate to columellate. The monocots also are supported by multiple characters: under MP they are characterized by boat-shaped grains with an elliptic polar outline (reversed in Zingiberales + Poales), under HB by very large size, echinate supratectal elements and granulate endexine. The grouping of Zingiberales + Poales is supported consistently across all methods by changes in symmetry, basic shape, and polar outline as previously described (Fig. 9) and, under HB optimization, three further characters: ectoaperture shape, annulus, and tectum sculpture. The other well-supported grouping is asterids + Caryophyllales, which are inferred to be characterized by supratectal elements and perforate tectum sculpture, as well as compound apertures and lack of an annulus in the HB optimization.

As discussed above, the clade perhaps best supported by pollen morphological synapomorphies is eudicots which share, at least under some methodologies, at least six linked character states (all reversed or modified in some terminal taxa). This agrees with, and expands upon, Donoghue and Doyle's (1989) tricolpate clade (note that there are of course many exceptions to tricolpate pollen as a diagnostic character, including tricolpate non-eudicots in Schisandraceae [Wang et al., 2009b] and *Nelumbo* Adans. [Banks et al., 2007], as well as eudicots with more or fewer apertures). Donoghue and Doyle did not specify whether they mean tricolpate with three meridional (equatorial) apertures sensu Erdtman (1943) or simply with three colpate apertures sensu Punt et al. (1994). In either case, based on our analyses, it is now possible to redefine the clade as isopolar, radially symmetrical, globose, and tri-zono-colpate. The independent evolution of tri-aperturate grains in Austrobaileyales (*Illicium oligandrum* Merr. & Chun), and also in Arecales (Harley & Dransfield, 2003) is confirmed by the fact that the three pores have a different developmental origin to those in most eudicots, being derived according to Garside's rule rather than Fischer's (Doyle et al., 1990). In fact, tri-aperturate grains derived following Garside's rule are observed to have evolved at least four times within angiosperms, in Austrobaileyales, Arecales, Proteales (Blackmore & Barnes, 1995), and Santalales (Maguire et al., 1974), as well as in the fossil pollen genus †*Aquilapollenites* Rouse, which may be associated with Santalales (Erdtman, 1971).

The eudicots comprise some 75% of all angiosperm species (Drinnan et al., 1994) and, given the several palynological synapomorphies now linked with the group, it is reasonable to ask whether pollen characters may in part be responsible for its diversity, perhaps by providing for more efficient production, transport, or fertilization of male gametes. It is also interesting to note that the origin, ca. 125 million years ago (Soltis et al., 2008), and subsequent diversification of eudicots was roughly coincident with that of certain groups of pollinating insects, such as bees, in the Early to Mid Cretaceous Period (e.g., Danforth et al., 2006).

FRAMEWORK FOR FUTURE DETAILED ANALYSES

To provide a framework for our future, detailed series of analyses of pollen evolution within angiosperms, we employed the most recent, comprehensive classification for the group (APG III, 2009), which comprises 70 orders, ordinal-level clades and unplaced taxa. To organize the orders and families of angiosperms as defined in this classification into rational groupings for analysis based on evolutionary relationship, we used the consensus tree published alongside this classification, which contains 63 orders and ordinal-level groups and was constructed from the results of several studies including Qiu et al. (1999), Graham and Olmstead (2000), Savolainen et al. (2000), Soltis et al. (2000), Bremer et al. (2002), Jansen et al. (2007), Moore et al. (2007), and Wang et al. (2009a). It is congruent with more recent studies such as those from Moore et al. (2010). This was modified (Fig. 10) to include four families—Icacinaceae, Oncothecaceae, Mettenusiaceae, and Vahliaceae—present in the APG III classification but not in the phylogeny presented alongside it (APG III, 2009). These are usually resolved in phylogenetic analyses to the lamiid clade (e.g., Soltis et al., 2007), although there is not strong support for any precise position.

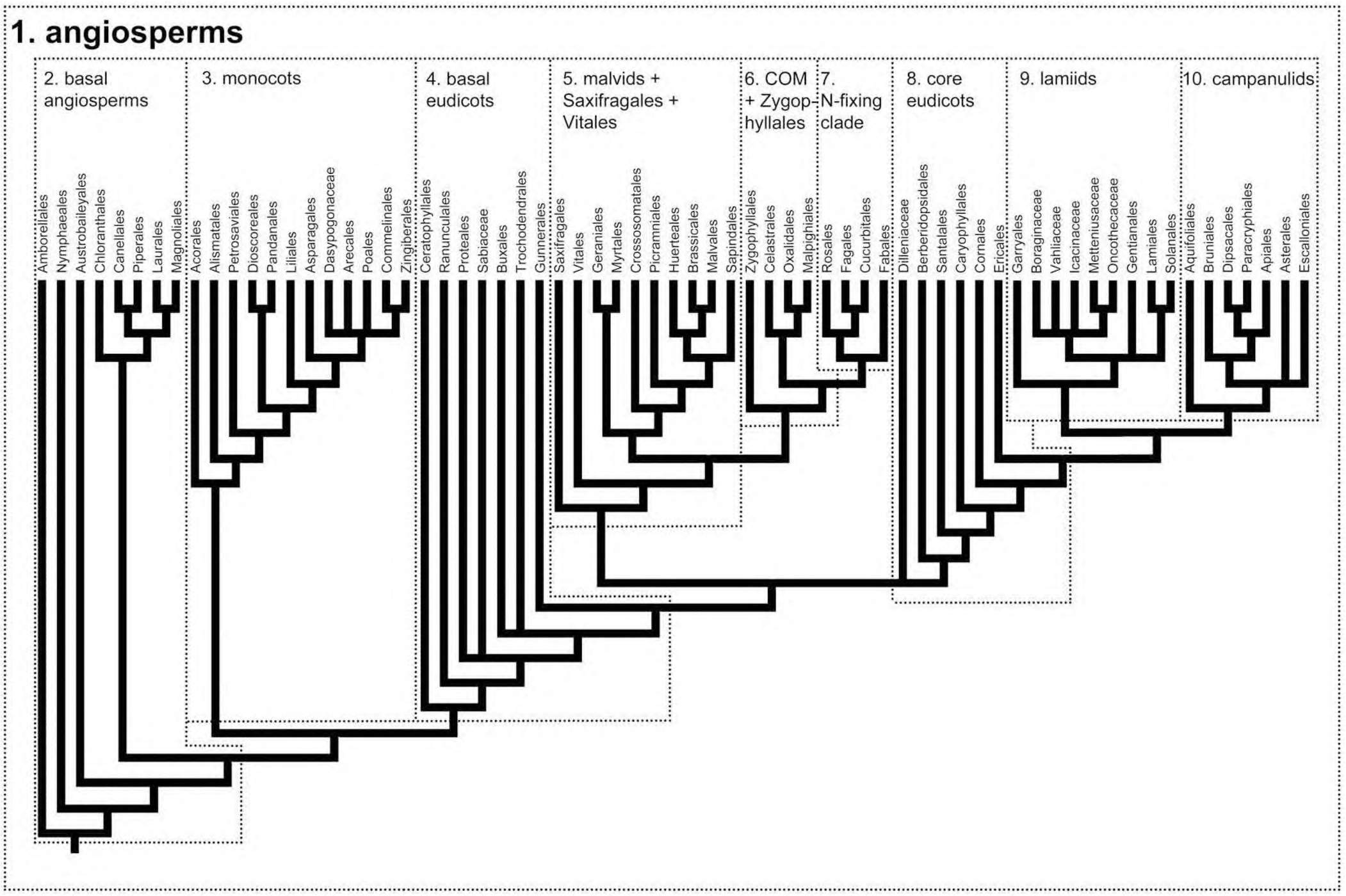


Figure 10. Framework phylogeny of angiosperm orders, divided into groups for subsequent analyses (for details see text). Formal and informal clade names taken from APG III (2009), Soltis et al. (1995), and Endress and Matthews (2006).

For the purposes of our study, they were placed with Boraginaceae as part of a polytomy at the base of the lamiids. This remains one of the most poorly resolved parts of the angiosperm tree, in need of more detailed phylogenetic analyses with larger amounts of data. Additional unplaced groups listed in the APG III classification (Apodanthaceae, Cynomoriaceae, *Gumillea* Ruiz & Pav., *Nicobariodendron* Vasudeva Rao & Chakrab., and *Petenaea* Lundell) were not included in our framework phylogeny since their likely affiliations were highly ambiguous; their pollen morphology will be analyzed in conjunction with their most likely relatives as discussed below.

We defined three criteria to divide the angiosperms into groups suitable for pollen evolutionary studies: (1) each group should be monophyletic or at least paraphyletic (never polyphyletic); (2) the groups should display similar ranges of pollen variability; and (3) they should be as similar as possible in size (number of species). Nine groups were defined (see Table 5; Fig. 10): basal angiosperms, monocots, basal eudicots + Ceratophyllales, malvids + Saxifragales + Vitales, COM (Celastrales, Oxalidales, Malpighiales) + Zygophyllaceae, the nitrogen-fixing clade, lamiids, campanulids, and other core eudicots.

A quick glance at Table 5 shows that these criteria were far from perfectly met but, most importantly, all groups provide a range of pollen variability that we consider feasible to study in a single paper, perhaps comparable to Nowicke and Skvarla's (1979) study of Centrospermae or Blackmore et al.'s (2009) on Compositae. Four of the nine groups defined are monophyletic (monocots, the nitrogen-fixing clade, lamiids, and campanuliids), with the remainder being paraphyletic. This is inevitable because, without sampling all orders individually, the removal of some monophyletic groups will always leave a paraphyletic subset. The criterion met least successfully was that of size, because this was considered subsidiary to the other two criteria. Thus, the size of the groups varies from ca. 6000 species (2% of angiosperms) in basal eudicots to 62,000 species (23%) in monocots. These size differences reflect the degree of pollen variation and depth of discussion required within the various groups. For instance, many monocots have relatively uniform pollen, while the pollen of basal eudicots is highly variable and likely to provide insights into the ancestral pollen type for both eudicots and core eudicots. To maintain consistent numbering across both groups and papers, including the present paper (Group 1, which deals with the angiosperms as a whole), the subsidiary groups are numbered 2 to 10 (Table 5; Fig. 10).

Group 2 (Table 5; Fig. 10; Lu et al., 2014), basal angiosperms, includes eight relatively small orders: Amborellales, Austrobaileyales, Canellales, Chloranthales, Laurales, Magnoliales, Nymphaeales, and Piperales, totaling 9335 species (3.5% of angiosperms). The group is paraphyletic, with a number of very long branches. This makes inference of ancestral states difficult and the use of model-based methods essential to allow multiple state changes on branches. With phylogenetic relationships still to some extent uncertain and implications for elucidating palynological traits underlying the diversification of the angiosperms as a whole, this is a crucial group.

Group 3 (Table 5; Fig. 10; Luo et al., in prep.), the monocots, is a monophyletic group comprising ca. 62,000 species (23% of angiosperms) in ca. 10 orders, including around 20,000 species in Poales and nearly 30,000 in Asparagales (22,500 within Orchidaceae). The relationships of most monocots are relatively clear, although the position and status of Dasypogonaceae are exceptions (APG III, 2009). The enigmatic genus *Ceratophyllum* L. (Ceratophyllaceae) is excluded from this group and placed in Group 4.

Group 4 (Table 5; Fig. 10; Zhang et al., 2015) contains the basal eudicots (Buxales, Gunnerales, Proteales, Ranunculales, Sabiaceae, and Trochodendrales) and *Ceratophyllum*, whose position as sister to eudicots is now relatively well supported (Jansen et al., 2007; Moore et al., 2007; APG III, 2009). Although small (ca. 6000 species, 2% of angiosperms), it is diverse in pollen morphology and may contain the answers to key questions underlying the explosive radiation of the eudicots. The positions of Buxales and Trochodendrales, and of Sabiaceae as sister to Proteales (and, therefore, its status as a separate order) remain uncertain (APG III, 2009).

Group 5 (Table 5; Fig. 10; Lu et al., 2015) is a fairly disparate, paraphyletic group of core eudicots, equating to the rosids excluding the monophyletic fabids (which are treated in Groups 6 and 7), with the addition of Saxifragales, which should perhaps be treated as a basal lineage of the rosids. Thus, Group 5 comprises Brassicales, Crossosomatales, Geraniales, Huerteales, Malvales, Myrtales, Picramniales, Sapindales, Saxifragales, and Vitales, a total of ca. 33,500 species (12.5% of angiosperms). Many of these lineages have only recently been defined and their relationships elucidated, e.g., Crossosomatales (APG III, 2009), Huerteales (Worberg et al., 2009), and Picramniales (Wang et al., 2009a). We also treat here the unplaced, isolated, monotypic genus *Petenaea*, identified in 1962 and limited to a small part of Central America, which is considered most likely a member of Malvales (Bayer et al., 1999; APG III, 2009), possibly close to Muntingiaceae. The pollen is reported as prolate, tricolporate, and microperforate (Bayer et al., 1999) but will merit further study to elucidate its relationships in more detail.

Groups 6 and 7 together make up the monophyletic fabids (Eurosid I). Group 6 (Table 5; Fig. 10; Luo et al., in prep.) comprises the monophyletic grouping of Celastrales, Malpighiales, and Oxalidales, collectively known as COM (Endress & Matthews, 2006; Zhu et al., 2007), plus Zygophyllales. The group contains ca. 20,000 species (ca. 7.5% of angiosperms). Here, we will also deal with two unplaced, isolated, monotypic genera, *Gumillea* and *Nicobariodendron*. The Peruvian species *G. auriculata* Ruiz. & Pav. was described in Cunoniaceae (Oxalidales) but is often considered a synonym of *Picramnia* Sw. (Picramniales; in Group 4) or placed in Simaroubaceae (Sapindales; in Group 5). It was maintained as separate by APG III (2009). Since it clearly belongs in one of the rosid groups, we will treat it here in Group 6 alongside Oxalidales. The pollen is little studied, although a very rough illustration is found in the work of Pavón (1798–1802: plate 245). *Nicobariodendron sleumeri* Vasudeva Rao & Chakrab. is possibly included in Celastraceae (Simmons, 2004). The species is endemic to the Nicobar Islands, very poorly collected, and the pollen has never been studied; it may provide very useful characters to help interpret the relationships of this enigmatic taxon but with such a limited distribution may be difficult to obtain.

Group 7 (Table 5; Fig. 10; He et al., in prep.), known as the nitrogen-fixing clade (Soltis et al., 1995, 1999), comprises Cucurbitales, Fabales, Fagales, and Rosales, together a monophyletic and strongly supported group in the recent analysis of Wang et al. (2009a). It contains ca. 31,000 species (11.5% of angiosperms). Here, we will also treat Apodanthaceae, a holoparasitic family of three genera, *Apodanthes* Poit., *Berlinianche* Vattimo, and *Pilostyles* Guill. (Mabberley, 2008). Apodanthaceae was previously included in Rafflesiaceae, from which it is now excluded (Blarer et al., 2000) and has since remained unplaced. Molecular data suggest a position either in Malvales or Cucurbitales (Nickrent et al., 2004). Cucurbitales now seems the more likely placement (Barkman et al., 2007; Filipowicz & Renner, 2010); thus, Apodanthaceae will be treated with Group 7. The pollen morphology of Apodanthaceae has been well studied (Takhtajan et al., 1985; Roubik & Moreno, 1991; Blarer et al., 2004) and, although highly variable within the family (ranging from inaperturate to tricolpate; Blarer et al., 2004), confirms its exclusion from Rafflesiaceae. However,

Table 5. Division of angiosperms (Group 1) into nine groups for subsequent palynological studies.

Group	Orders and ordinal-level clades contained	Approximate number of species	Approximate % angiosperms
2. Basal angiosperms	Amborellales, Austrobaileyales, Canellales, Chloranthales, Laurales, Magnoliales, Nymphaeales, Piperales	9335	3.5
3. Monocots	Acorales, Alismatales, Arecales, Asparagales, Commelinales, Dasypogonaceae, Dioscoreales, Liliales, Pandanales, Petrosaviales, Poales, Zingiberales	62,000	23
4. Basal eudicots + Ceratophyllales	Buxales, Ceratophyllales, Gunnerales, Proteales, Ranunculales, Sabiaceae, Trochodendrales	6000	2
5. Malvids + Saxifragales + Vitales	Brassicales, Crossosomatales, Geraniales, Huerteales, Malvales, Myrtales, Picramniales, Sapindales, Saxifragales, Vitales	33,500	12.5
6. COM + Zygophyllales	Celastrales, Malpighiales, Oxalidales (COM; Endress & Matthews, 2006; Zhu et al., 2007), Zygophyllales	20,000	7.5
7. Nitrogen-fixing clade	Cucurbitales, Fabales, Fagales, Rosales	31,000	11.5
8. Other core eudicots	Berberidopsidales, Caryophyllales, Cornales, Dilleniaceae, Ericales, Santalales	25,500	9.5
9. Lamiids	Boraginaceae, Garryales, Gentianales, Icacinaceae, Lamiales, Metteniusaceae, Oncothecaceae, Solanales, Vahliaceae	47,000	17.5
10. Campanuliids	Apiales, Aquifoliales, Asterales, Bruniales, Dipsacales, Escalloniales, Paracryphiales	33,000	12.5

pollen characters of Apodanthaceae have not been considered in the context of related families, and it is hoped further study might improve our understanding of the relationships of this family.

Group 8 (Table 5; Fig. 10; Wang et al., in prep.) comprises a paraphyletic grade of core eudicots that subtend the sister grouping of lamiids and campanulids. Together, the Berberidopsidales, Caryophyllales, Cornales, Dilleniaceae, Ericales, and Santalales include ca. 25,500 species (9.5% of angiosperms) with some interesting palynological features (such as pollen dispersed in tetrads in many Ericaceae). Cynomoriaceae, a holoparasitic, monogeneric family of one (Mabberley, 2008) or two species (Nickrent et al., 2005) from eastern Asia and the Mediterranean, has been placed variously in Saxifragales (Nickrent et al., 2005) or Santalales (Jian et al., 2008), sometimes under Balanophoraceae (e.g., Cronquist, 1981). Saxifragales and Santalales are quite separate in recent classifications (APG III, 2009) and fall into two different groups in our framework. Here, we will treat Cynomoriaceae in Group 8 alongside Santalales, as suggested (albeit poorly supported) by the most recent molecular results (Jian et al., 2008). Pollen of *Cynomorium* L. has not been studied since 1975, and previous studies have focused on pollen development rather than pollen grain morphology (Steindl, 1945; Terekhin et al., 1975), so further studies would be highly beneficial.

Groups 9 (lamiids) and 10 (campanulids) are relatively well-supported, monophyletic sister groups. The lamiids (Group 9; Table 5; Fig. 10; Wortley et al., in prep.) comprise ca. 47,000 species (17.5% of angiosperms), including Boraginaceae, Garryales, Gentianales, Icacinaceae, Lamiales, Metteniusaceae, Oncothecaceae, Solanales, and Vahliaceae. Pollen morphology within this group is highly variable, e.g., in Acanthaceae (Lamiales; Scotland, 1992). A number of families within this group (Boraginaceae, Icacinaceae, Metteniusaceae, Oncothecaceae, and Vahliaceae) remain of uncertain position and status (APG III, 2009). Again, it is hoped that pollen morphological study may shed light on their relationships.

The campanulids (Group 10; Table 5; Fig. 10; La et al., in prep.) include Apiales, Aquifoliales, Asterales, Bruniales, Dipsacales, Escalloniales, and Paracryphiales, a total of ca. 33,000 species (12.5% of angiosperms). Within the group, the positions of Escalloniales and Bruniales remain somewhat uncertain (APG III, 2009). More than half the group is included in a single family, Asteraceae (ca. 23,600 species), a family that has already been subject to detailed study of pollen morphological characters, which were found to be very useful for defining and diagnosing clades within it (Blackmore et al., 2009).

The methodology for treating each of these nine groups will be based on the results of the present study. Thus, base phylogenies will provide broad coverage of all orders and most families and will be based on molecular genetic data, ideally multiple loci, analyzed using model-based methods and presented with branch lengths. If, as in the present study, any unstable nodes are confined to morphologically homogeneous regions of the tree, a single phylogenetic estimate may be used as a base. However, in cases where there are significant controversies between topologies, it will be prudent to test multiple phylogenies. For paraphyletic groups, the overview phylogeny will contain representatives of the segregate taxa that render the study group paraphyletic, so that the phylogeny as a whole is monophyletic for the purposes of the study. For groups where a suitable overview phylogeny cannot be found, the appropriate section of a broader study such as the multi-gene analyses from Soltis et al. (2007, 2011) may be used; these include representatives of all orders in APG III (2009). Although it might be possible to build supertrees from more densely sampled smaller trees, Torices (2010) has shown that errors in branch length reconstruction on supertrees can lead to differences in the inference of character states, at least with ML. Additional, more comprehensive phylogenies may be selected for the constituent orders, sampling at least to family and possibly to generic level depending on the size and variability of the group, and coding for more specialized characters, the nature of which will depend on the palynological makeup of each particular group.

We have shown that coding strategy can have a significant effect upon ancestral character state reconstruction, perhaps most significant in characters that are informative at higher taxonomic levels but also variable at lower levels (i.e., within terminal taxa). For future papers, we recommend the use of democratic coding, which provides a good compromise between levels of polymorphism and missing data. However, the impacts of coding, as seen in this study, could be minimized by dense sampling and model-based optimization methods; if for this reason coding strategy is no longer considered to be an issue, the simpler species exemplar method may be used. Because optimization method can also have a significant effect upon ancestral character state reconstruction, probably due to differing models of evolution, future studies should employ at least one method with a simple optimization model (MP or ML) and one with a complex model (HB) for comparison.

Conclusions

The APG classification (APG III, 2009) is fast becoming the baseline of choice for robust evolutionary investigations of all kinds (for a recent example, see Hawkins et al., 2011). Our APG-based preliminary analysis of pollen characters in angiosperms immediately suggests diagnostic and synapomorphic characters for key monophyletic groups including angiosperms themselves and eudicots, as well as potential hypotheses of evolutionary process. In combination with recent successful family-level studies (e.g., Blackmore et al., 2009), it suggests that further, detailed analyses should be a priority for palynological research, to facilitate diagnosis of groups, indicate potential relationships for unplaced or unstable taxa, and uncover correlations with evolutionary/ecological events. The identification of key ancestral state combinations may also facilitate more accurate placement of fossil pollen grains in a phylogenetic context, supporting the dating and calibration of phylogenetic trees. Figure 10 suggests a number of taxa for which pollen morphology will be of particular interest in determining evolutionary patterns, such as many basal angiosperms, Acorales (the first branching clade of monocots), Ceratophyllales (sister to eudicots), Ranunculales (the first branching lineage of eudicots), Trochodendrales (the potential sister group to core eudicots), Gunnerales (the first branching lineage in core eudicots), Saxifragales (sister to rosids), Vitales (the first branching lineage in rosids), and many of the taxa in Group 8 which are potential sister groups to asterids or larger clades. Some of these, including Acorales (Wang et al., 1995) and Gunnerales (Wanntorp et al., 2004), have been relatively little studied.

Our work provides a reference for future palynological and systematic studies, a stimulus for angiosperm-wide analysis of other morphological character sets, and a methodological protocol for such studies to proceed. In terms of the latter, this paper shows that the results of ancestral trait optimization can be unstable when variables such as topology, coding, and evolutionary model are changed. Tree topology had little effect upon ancestral character state inference where discrepancies involved only nodes without inferences of character state change. Coding strategy can have a significant effect upon ancestral character state inference, probably due to differing levels of missing data and polymorphism between coding strategies. This effect is perhaps most significant in those characters that are informative at higher taxonomic levels but also variable at lower levels (i.e., within

terminal taxa). For such characters, species exemplar coding results in a greater number of inferred character state changes on internal branches within the phylogeny, while comprehensive coding results in fewer such changes. Finally, optimization method can also have a significant effect upon ancestral character state optimization. Until it becomes clear which models and priors are most appropriate, comparison of multiple methods with different assumptions is the most prudent approach to robust optimization.

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APPENDIX 1. Literature sources used for palynological data, with angiosperms listed alphabetically by taxonomic order.

Gymnosperm outgroups (Wodehouse, 1935; Erdtman, 1943, 1957; Walker, 1974; Millay & Taylor, 1976; Nilsson et al., 1977; Audran & Masure, 1978; Sahashi & Ueno, 1986; Dehgan & Dehgan, 1988; Médus et al., 1988; Pacini et al., 1999; Zhang et al., 2000).

Angiosperm orders. ACORALES (Erdtman, 1952; Grayum, 1992; Rudall et al., 1997; Furness & Rudall, 2003; Sage et al., 2009; Wu et al., 2010); AMBORELLALES (Erdtman, 1952; Walker, 1976; Dahlgren & Clifford, 1982; Sampson, 1993, 2000; Doyle, 2009); APIALES (Cerceau-Larrival & Roland-Heydacker, 1976; Punt, 1984; Huysmans et al., 1998; Wen & Nowicke, 1999); ARECALES (Sowunmi, 1972; Ferguson & Harley, 1993; Martins et al., 2003); ASPARAGALES (Erdtman, 1952; Schill & Pfeiffer, 1977; Burns-Balogh, 1983; Alvarez & Köhler, 1987; Chung & Jones, 1989; Zavada, 1990; Freudenstein & Rasmussen, 1999; Chase et al., 2000); ASTERALES (Erdtman, 1952; Horner & Pearson, 1978; Blackmore, 1984; Dunbar, 1984; Dehgan & Dehgan, 1988; Vaissière & Vinson, 1994; Gustafsson et al., 1997; Hansen, 1997; Perveen & Qaiser, 1999; Lundberg & Bremer, 2003; Polevova, 2006); AUSTROBAILEYALES (Erdtman, 1952; Liu & Yang, 1989; Takahashi, 1994; Sampson, 2000; Yao et al., 2004; Wang et al., 2009b); BRASSICALES (Erdtman et al., 1969; Nilsson et al., 1977; Lahham & Al-Eisawi, 1987; Tomb, 1999; Perveen et al., 2004; Khan, 2005; Diethart et al., 2007; Suzuki et al., 2008; Santos, 2010); BUXALES (Köhler & Brückner, 1982; Nowicke & Skvarla, 1984; Punt & Marks, 1991; Brückner, 1993; Goldberg & Alden, 2005); CANELLALES (Erdtman, 1952; Praglowski, 1979; Acosta Castellanos & Vilela, 1998; Doyle, 2000; Sampson, 2000); CARYOPHYLLALES (Wodehouse, 1935; Erdtman, 1952; Nyananyo, 1992; Behnke, 1997; Olvera et al., 2006); CHLORANTHALES (Walker & Walker, 1984; Eklund et al., 2004); CUCURBITALES (Erdtman, 1952; Saad, 1964; Matthews & Endress, 2004; Perveen & Qaiser, 2008a); DIOSCOREALES (Erdtman, 1952; Zavada, 1983; Schols et al., 2003, 2005); FABALES (Lesins & Lesins, 1963; Ferguson & Skvarla, 1981; Crompton & Grant, 1983; Perveen & Qaiser, 1998a, 1998b, 1998c; Koti et al., 2004; Claxton et al., 2005); GENTIANALES (Chinnappa & Warner, 1981, 1982; Stoffelen et al., 1997; Vinckier et al., 2000); GERANIALES (Larson, 1966; Stafford & Gibby, 1992; Shehata, 2008); LAMIALES (Erdtman, 1952; Lee & Park, 1982; Punt et al., 1991; Bove, 1993); LAURALES (Erdtman, 1952; Walker, 1976; Sampson, 2000); MAGNOLIALES (Wodehouse, 1935; Erdtman, 1952; Walker, 1974, 1976; Wei & Wu, 1993); MALPIGHIALES (Erdtman, 1952; Presting, 1965; Larson, 1966; Rowley & Erdtman, 1967; Nilsson et al., 1977; Nowicke, 1994; Lobreau-Callen & Cervera, 1997; Amela García et al., 2002; Hansen et al., 2006; Wurdack & Davis, 2009); MALVALES (Bronckers & Horvat, 1963; Beasley, 1975; Vaissière & Vinson, 1994; Lan et al., 1995; Nilsson et al., 1996); MYRTALES (Pike, 1955; Skvarla et al., 1976, 1978; Patel et al., 1984; Levin et al., 2003; Punt et al., 2003b; Eliseu & Dinis, 2008); NYMPHAEALES (Wodehouse, 1935; Erdtman, 1943, 1952; Rowley, 1967; Jones & Clarke, 1981; Sampson, 2000; Doyle, 2005; Padgett, 2007; Borsch et al., 2008; Remizowa et al., 2008; Zhou & Fu, 2008); PIPERALES (Erdtman, 1952; Lei & Liang, 1998; Sampson, 2000; Mathew & Mathew, 2001); POALES (Wodehouse, 1935; Erdtman, 1943, 1952; Skvarla & Larson, 1966; Christensen et al., 1972; Punt, 1975; Ladd, 1977; Nilsson et al., 1977; Köhler & Lange, 1979; Zavada, 1983; El-Ghazaly & Jensen, 1986, 1990; Chaturvedi et al., 1994, 1998; Chaturvedi & Datta, 2001; Dönmez & Işik, 2008; Mbagwu et al., 2008); PROTEALES (Wodehouse, 1935; Erdtman, 1943; Denk & Tekleva, 2006); RANUNCULALES (Erdtman, 1952; Nowicke & Skvarla, 1981; Clarke et al., 1991; Huysmans et al., 1998; Furness et al., 2007); ROSALES (Erdtman, 1952; Punt & Malotaux, 1984; Pehlivan, 1987); SAPINDALES (Erdtman, 1952; Grant et al., 2000); SOLANALES (Erdtman, 1952; Godwin et al., 1967; Godwin, 1968; Sengupta, 1972; Sharma, 1974; Punt & Monnabrands, 1977; Cresti et al., 1985; Trigo, 1992; Huysmans et al., 1998; Tellería & Daners, 2003; Al-Wadi & Lashin, 2007; Zhang et al., 2009); VITALES (Erdtman, 1952; Sharma, 1967; Zavada, 1983; Inceoglu et al., 2000; Punt et al., 2003a; Perveen & Qaiser, 2008b).

APPENDIX 2. Genera for which palynological data were not available for the specific taxa sampled by Jansen et al. (2007) in the molecular phylogeny, giving the closely related taxon used in their place, with angiosperms listed alphabetically by genus.

Gymnosperm outgroups: *Cycas revoluta* Thunb. data used for *C. micronesica* K. D. Hill; *Pinus scopulorum* (Engelm.) Lemmon and *P. sylvestris* L. for *P. thunbergii* Parl.

Angiosperm genera: *Acorus gramineus* Sol. ex Aiton data used for both *A. americanus* (Raf.) Raf. (Acorus.ame) and *A. calamus* L. (Acorus.cal); *Brassica campestris* L., *Brassica napus* L., and *Brassica tournefortii* Gouan for *Brassica rapa* L.; *Buxus sempervirens* L. for *Buxus microphylla* Siebold & Zucc.; *Campanula latifolia* L. and *Campanula strigosa* Vahl for *Trachelium caeruleum* L. (aff. *Campanula* L. sensu Roquet et al., 2008); *Chloranthus inconspicuus* Sw. and *Chloranthus japonicus* Siebold for *Chloranthus spicatus* (Thunb.) Makino; *Citrus limon* (L.) Osbeck and *Citrus medica* L. for *Citrus sinensis* (L.) Osbeck; *Coffea congensis* A. Froehner for *Coffea arabica* L.; *Cucumis melo* L. for *Cucumis sativus* L.; *Dioscorea polygonoides* Humb. & Bonpl. ex Willd. for *Dioscorea elephantipes* (L'Hér.) Engl.; *Drimys winteri* J. R. Forst. & G. Forst. for *Drimys granadensis* L. f.; *Elaeis guineensis* Jacq. for *E. oleifera* (Kunth) Cortés; *Illicium fargesii* Finet & Gagnep., *I. floridanum* J. Ellis, and *I. religiosum* Siebold & Zucc. for *I. oligandrum* Merr. & Chun; *Jasminum fruticans* L. and *J. humile* L. for *J. nudiflorum* Lindl.; *Medicago lupulina* L. for *Medicago truncatula* Gaertn.; *Morus alba* L. and *Morus nigra* L. for *Morus indica* L.; *Musa coccinea* Andrews for *Musa acuminata* Colla; *Nicotiana alata* Link & Otto, *Nicotiana glauca* Graham, and *Nicotiana tabacum* L. data used for all three species *Nicotiana sylvestris* Speg. & S. Comes (Nicotiana.syl), *Nicotiana tabacum* (Nicotiana.tab), and *Nicotiana tomentosa* (Nicotiana.tom), depending on the character (not all characters documented in the literature for all species); *Nymphaea colorata* Peter for *Nymphaea alba* L.; *Oenothera biennis* L. and *O. maysillesii* Munz for *O. elata* Kunth; *Passiflora caerulea* L. and *Passiflora suberosa* L. for *Passiflora biflora* Lam.; *Pelargonium grandiflorum* Willd. for *Pelargonium* × *hortorum* L. H. Bailey; *Phalaenopsis lueddemanniana* Rchb. f. (Orchidaceae) and data ascribed to other Vandeae for *Phalaenopsis aphrodite* Rchb. f.; *Piper majusculum* Blume (Piperaceae), *Piper nigrum* L., and *Piper polysyphonum* C. DC. for *Piper cenocladum* C. DC.; *Populus tremula* L. for *Populus trichocarpa* Torr. & A. Gray; *Ranunculus repens* L. for *R. macranthus* Scheele; *Scaevola taccada* (Gaertn.) Roxb. for *Scaevola aemula* R. Br.; *Solanum lycopersicum* L., *Solanum lysimachioides* Wall., and *Solanum macracanthum* A. Rich. data used for all three species *Solanum bulbocastaneum* Dunal (Solanum.bul), *Solanum lycopersicum* L. (Solanum.lyc), and *Solanum tuberosum* L. (Solanum.tub), depending on the character (not all characters documented in the literature for all species); *Vitis sylvestris* Blume for *V. vinifera* L.; *Yucca elephantipes* Hort. ex Regel for *Y. schidigera* Ortgies.

APPENDIX 3. List of pollen morphological characters and character states used in the four matrices studied. C, comprehensive method of coding; D, democratic method; E, exemplar method; E(NP), exemplar method with polymorphic data points treated as missing data.

1. Dispersal unit
   C: 0, monad; 1, dyad; 2, permanent tetrad; 3, polyad; 4, pollinia.
   D: 0, monad.
   E, E(NP): 0, monad; 1, permanent tetrad; 2, pollinia.
2. Polarity
   C, D, E, E(NP): 0, apolar; 1, heteropolar; 2, isopolar (including subisopolar).
3. Symmetry in polar view
   C, D, E, E(NP): : 0, bilateral; 1, radial.
4. Basic shape
   C, D, E, E(NP): 0, boat-shaped; 1, globose.
5. Shape class
   C: 0, peroblate; 1, oblate; 2, subspheroidal; 3, prolate; 4, perprolate.
   D: 0, oblate; 1, subspheroidal.
   E: 0, oblate; 1, subspheroidal; 2, prolate; 3, perprolate.
   E(NP): 0, oblate; 1, subspheroidal; 2, prolate.
6. Outline in polar view
   C: 0, elliptic; 1, circular; 2, polygonal; 3, lobate; 4, concave-polygonal.
   D, E, E(NP): 0, elliptic; 1, circular; 2, polygonal; 3, lobate.
7. Size
   C, E, E(NP): 0, very small; 1, small; 2, medium; 3, large; 4, very large.
   D: 0, small; 1, medium; 2, large.
8. Aperture number
   C, E, E(NP): 0, zero; 1, one; 2, two; 3, three; 4, four to six; 5, seven to 12; 6, many.
   D: 0, zero; 1, one; 2, three; 3, many.
9. Aperture position
   C, D, E, E(NP): 0, distal; 1, equatorial; 2, global.
10. Aperture structure
    C, D, E, E(NP): 0, simple; 1, compound.
11. Ectoaperture shape
    C: 0, porate; 1, colpate; 2, zonate; 3, spiral; 4, syncolpate.
    D: 0, porate; 1, colpate.
    E: 0, porate; 1, colpate; 2, spiral; 3, syncolpate.
    E(NP): 0, porate; 1, colpate; 2, syncolpate.
12. Aperture membrane ornamentation
    C, D, E, E(NP): 0, smooth; 1, granulate.
13. Operculum
    C, D, E, E(NP): 0, absent; 1, present.
14. Annulus
    C, E, E(NP): 0, absent; 1, costate; 2, aspidate; 3, vestibulate.
    D: 0, absent; 1, costate; 2, aspidate.
15. Exine differentiation
    C, D, E, E(NP): 0, absent; 1, present.
16. Supratectal elements
    C, D, E, E(NP): 0, absent; 1, present.
17. Supratectal element shape
    C, D: 0, verrucate; 1, echinate; 2, gemmate; 3, pilate.
    E, E(NP): 0, verrucate; 1, echinate; 2, gemmate.
18. Supratectal element size
    C, D, E, E(NP): 0, smaller than 1 μm; 1, greater than 1 μm.
19. Tectum sculpture
    C, E: 0, imperforate; 1, areolate; 2, *Croton*-pattern; 3, *Amborella*-type; 4, fossulate; 5, foveolate; 6, perforate; 7, reticulate; 8, rugulate; 9, striate; A, striato-reticulate.
    D: 0, imperforate; 1, *Croton*-patterned; 2, *Amborella*-type; 3, foveolate; 4, perforate; 5, reticulate; 6, striate.
    E(NP): 0, imperforate; 1, areolate; 2, *Croton*-patterned; 3, *Amborella*-type; 4, fossulate; 5, foveolate; 6, perforate; 7, reticulate; 8, rugulate; 9, striate.
20. Infratectum structure
    C, E, E(NP): 0, alveolate; 1, columellate; 2, granulate.
    D: 0, alveolate; 1, columellate.
21. Number of infratectum layers
    C, D, E, E(NP): 0, one; 1, two.
22. Foot layer
    C, D, E, E(NP): 0, absent; 1, present.
23. Endexine

C, D, E, E(NP): 0, absent; 1, present.
24. Endexine type
C, D, E, E(NP): 0, compact; 1, lamellar; 2, granular/spongy.
25. Endexine extent
C, D, E, E(NP): 0, continuous; 1, discontinuous; 2, apertural.
26. Cavea
C, D, E, E(NP): 0, absent; 1, present.
27. Internal foramina
C, D, E, E(NP): 0, absent; 1, present.
28. Microchannels
C, D, E, E(NP): 0, absent; 1, present.
29. Exine folding
C, D, E, E(NP): 0, non-lophate; 1, lophate.
30. Viscin threads
C, D, E, E(NP): 0, absent; 1, present.
31. Orbicules
C, D, E, E(NP): 0, absent; 1, present.

APPENDIX 4. Testing the impact of choice of priors for HB inference.

Multiple types of prior distribution were compared for two test characters, using the species exemplar matrix (polymorphic data points treated as missing data): Character 7 (pollen grain size), a multi-state character expected to display little structure with respect to the phylogenetic hypothesis; and Character 10 (aperture structure), a binary character expected to show clear structure with respect to the phylogenetic hypothesis. The priors tested were: uniform distribution; beta, exponential, and gamma distributions with parameters as indicated by the transition rates from the empirical Bayesian analysis; and a distribution generated by the reversible-jump hyperprior (rjhp) mechanism, as recommended in the BayesTraits manual (Pagel & Meade, 2006).

With both characters, the uniform prior distribution produced clearly unrealistic posterior distributions of inferred ancestral states, in which all states were almost equally probable at every internal node. This implies that a uniform prior distribution can dominate the results of small datasets, as suggested by Pagel and Meade (2006). Furthermore, likelihood values (-Lh) were much lower (e.g., for Character 7, −79.8 compared with −46 to −50) than with alternative prior distributions for the same data. Priors set using the rjhp, and with beta, exponential, and gamma priors using parameters derived from the EB analysis, all produced similar results in terms of inferred ancestral states.

For Character 7 (pollen grain size), the posterior distributions obtained using gamma- and exponentially distributed priors were most similar to one another; the posterior distributions obtained using the rjhp method were similar to those seen using EB; those obtained using beta-distributed priors were different from the other methods and tended to produce more ambiguous results, rather similar to using a uniform prior. The highest -Lh value was obtained using the rjhp approach. For Character 10 (aperture structure), the rjhp mechanism produced results most similar to those obtained with MP and ML, while the beta, exponential, and gamma prior distributions produced results very similar to one another, with slightly lower -Lh values than the rjhp mechanism.

Based on these results, in which beta-, exponential-, and gamma-distributed priors, as well as priors generated using the rjhp, produced broadly similar results, we chose to use the rjhp mechanism as recommended by Pagel and Meade (2006), specifying an exponential or gamma prior distribution where this suits the data for each particular character. The optimal parameters obtained for HB (continuous-time Markov model; aiming for an average acceptance rate of ca. 30% during rjhp optimization) ranged from gamma to exponential distributions, with seed means from 0 or 1 (Character 13) to 0 to 150 (Character 27), seed variances (for gamma distributions only) from 0 to 10 (Character 2) to 0 to 100 (Characters 19 and 26), and rate deviations from 5 (Character 15) to 180 (Character 17).

Appendix 5. Morphological matrices analyzed in this study (for simplicity, taxon names used are the same as those in Jansen et al. [2007] throughout).

Comprehensive method

Acorus.ame	0100-01100110010--{56}1011100??000
Acorus.cal	0100-01100110010--{56}1011100??000
Amborella	010{01}2{01}{12}{01}00{01}{01}0011103101{01}02000000
Anethum	02{01}1{234}{12}{123}{234}11110{012}10--{6789A}10110000000{01}
Arabidopsis	0{02}{01}1{123}{123}{1234}{0234}1{01}{14}{01}{01}{02}1{01}{01} 0{67A}101{01}10000000
Atropa	{02}{02}11{123}{0123}{1234}{3456}{12}{01}{01}{01}0{023}1{01}{0123}{01} {0567A}10111000000{01}
Brassica	0{02}{01}1{123}{123}{123}{0234}1{01}{14}{01}{01}{02} 1{01}{01}0{67A}101{01}10000000
Buxus	0{02}1121{12}{36}{12}{01}{01}0001{01}{01}0{27}101110000000
Calycanthus	{02}{02}{01}121{123}{02}1011001{01}{012}{01}{679}101111000000
Chloranthus	0{012}{01}{01}2{13}{12}{014}{01}011{01}010--7101110000000
Citrus	{02}{12}11{01234}{12}{123}{2345}1{01}{014}1{01} {012}1{01}{123}{01}{079A}1011?00??000
Coffea	{02}{12}{01}1{0123}{12}{1234}{012346}{12}{01}{0123}{01}0{02}1{01} {012}{01}{45679A}1011?00??000
Cucumis	{02}2{01}1{01234}{0123}{1234}{2346}1{01}{014} 10{023}1{01}11{5679}1011??0??000
Cycas	0100-{01}{123}100100010--{456}001110000000
Daucus	02{01}1{234}{12}{123}{234}11110{012}10--{6789A}10110000000{01}
Dioscorea	01{01}{01}-{01}{23}{1234}{01}0{01}1001{01}10{67}101120000000
Drimys	{02}1{01}11{12}2100{01}00{02}10--{67}101110000000
Elaeis	01001{02}{123}{12}{01}0{01}0{01}01{01}{01}{01}{67}101???0??000
Eucalyptus	{02}2{01}1{0123}{02}{01234}{234}1{01}{0134}{01}0{02}1{01}{02}0{01689}10 {01}1000000{01}0
Ginkgo	010{01}202100{01}0001{01}10{89}00111000?000
Glycine	{023}211{0123}{123}{1234}{23456}111{01}0{013}1 {01}10{0479A}10{01}1?0000000
Gossypium	{02}{02}{01}1{123}{12}{1234}{23456}{12}{01}{014}1{01}{012}1 {01}{01}{01}{267A}1011{02}{01}000000
Helianthus	{02}211{0123}{12}{1234}{2345}1{01}{014}{01}{01}{02}1{01}1{01} {045679}1{01}{01}1{01}0 {01}{01}0{01}00
Hordeum	{02}{012}{01}1{123}{12}{1234}{012346}00{0123}{01} {01}{12}1{01}{01}{01}{15789} 101{01}2000100{01}
Illicium	0{12}{01}1{12}{12}{123}{12345}{01}0{014}{01}001120{57}101100000000
Ipomoea	{02}{02}11{123}{0123}{1234}{3456}{12}{01}{01}{01}0{023}1{01}{0123}{01} {05679A}10111000000{01}
Jasminum	{02}{02}{01}1{01234}{123}{1234}{0123456}{12}{01}{01234} {01}{01}{02}1{01}{01}{01} {1679}10{01}1?00??000
Lactuca	{02}211{0123}{12}{1234}{2345}1{01}{014}{01}{01}{02}1{01}1{01}{045679}1 {01}{01}1{01}0{01}{01}0{01}00
Liriodendron	{02}{012}{01}{01}20{234}{0123}{01}0{12}1001{01}00{678}101110000000
Lotus	{023}211{0123}{123}{1234}{23456}111{01}0{013} 1{01}10{04789A}10{01}1?0000000
Manihot	{012}{02}11{01234}{0123}{1234}{03456}{12}{01}{0124}1 {01}{02}1{01}{012}{01}{02679}10111{01}000000
Medicago	{023}211{0123}{123}{1234}{23456}111{01}0{013} 1{01}10{04789A}10{01}1?0000000
Morus	{02}2{01}1{123}{01234}{123}{2345}1{01}{014} 1{01}{02}1{01}1{01}{1789}1011?0000000
Musa	0{01}1121{234}{0156}{02}0{01}?000{01}{12}{01}--------??000
Nandina	{02}{012}11{234}{123}{123}{013456}1{01}{013}{01} {01}01{01}{01}{01}{679A}10111{02}00000{01}

Appendix 5. Continued.

Nicotiana.syl	{02}{02}11{123}{0123}{1234}{3456}{12}{01}{01}{01}0{023}1{01}{0123}{01}{05679A}10111000000{01}
Nicotiana.tab	{02}{02}11{123}{0123}{1234}{3456}{12}{01}{01}{01}0{023}1{01}{0123}{01}{05679A}10111000000{01}
Nicotiana.tom	{02}{02}11{123}{0123}{1234}{3456}{12}{01}{01}{01}0{023}1{01}{0123}{01}{05679A}10111000000{01}
Nuphar	{02}10{01}2{012}{123}{01234}{01}0{12}1{01}01{01}{012}{01}{069}{12}01{01}1?000000
Nymphaea	{02}10{01}2{012}{123}{01234}{01}0{12}1{01}01{01}{012}{01}{069}{12}01{01}1?000000
Oenothera	{02}2{01}1{0123}{02}{01234}{234}1{01}{0134}{01}0{02}1{01}{02}0{01689}10{01}1000000{01}0
Oryza	{02}{012}{01}1{123}{12}{1234}{012346}00{0123}{01}{01}{12}1{01}{01}{01}{15789}101{01}2000100{01}
Panax	02{01}1{234}{12}{123}{234}11110{012}10--{6789A}10110000000{01}
Passiflora	{012}{02}11{01234}{0123}{1234}{03456}{12}{01}{0124}1{01}{02}1{01}{012}{01}{02679}10111{01}000000
Pelargonium	0{02}11{23}{123}{234}{036}{12}{01}{01}{01}001{01}{01}{01}{79A}101{01}?0001000
Phalaenopsis	{024}{012}{01}{01}2{01}{01234}{01236}00{013}1{01}01{01}{01}0{05679}1001?0000000
Pinus	0{01}{01}{01}2{01}{123}{01}00{01}{01}001{01}{12}0{1567}{02}01110000000
Piper	{02}1012{01}{0123}{0123}00{01}1001{01}{01}1{178}{02}011100??000
Platanus	02{01}1{012}{0124}{123}{234}1{01}{01}1001{01}{01}{01}{57}101100000000
Populus	{012}{02}11{01234}{0123}{1234}{03456}{12}{01}{0124}1{01}{02}1{01}{012}{01}{02679}10111{01}000000
Ranunculus	{02}{012}11{234}{123}{123}{013456}1{01}{013}{01}{01}01{01}{01}{01}{679A}10111{02}00000{01}
Saccharum	{02}{012}{01}1{123}{12}{1234}{012346}00{0123}{01}{01}{12}1{01}{01}{01}{15789}101{01}2000100{01}
Scaevola	{02}211{0123}{12}{1234}{2345}1{01}{014}{01}{01}{02}1{01}1{01}{045679}1{01}{01}1{01}0{01}{01}0{01}00
Solanum.bul	{02}{02}11{123}{0123}{1234}{3456}{12}{01}{01}{01}0{023}1{01}{0123}{01}{05679A}10111000000{01}
Solanum.lyc	{02}{02}11{123}{0123}{1234}{3456}{12}{01}{01}{01}0{023}1{01}{0123}{01}{05679A}10111000000{01}
Solanum.tub	{02}{02}11{123}{0123}{1234}{3456}{12}{01}{01}{01}0{023}1{01}{0123}{01}{05679A}10111000000{01}
Sorghum	{02}{012}{01}1{123}{12}{1234}{012346}00{0123}{01}{01}{12}1{01}{01}{01}{15789}101{01}2000100{01}
Spinacia	{02}{02}11{123}{12}{1234}{3456}{12}{01}{014}1{01}{02}1{01}{01}0{567}1011??0{01}?000
Trachelium	{02}211{0123}{12}{1234}{2345}1{01}{014}{01}{01}{02}1{01}1{01}{045679}1{01}{01}1{01}0{01}{01}0{01}00
Triticum	{02}{012}{01}1{123}{12}{1234}{012346}00{0123}{01}{01}{12}1{01}{01}{01}{15789}101{01}2000100{01}
Typha	{02}{012}{01}1{123}{12}{1234}{012346}00{0123}{01}{01}{12}1{01}{01}{01}{15789}101{01}2000100{01}
Vitis	0211{23}{123}{12}{34}11110110--{78}1011??000000
Yucca	{024}{012}{01}{01}2{01}{01234}{01236}00{013}1{01}01{01}{01}0{05679}1001?0000000
Zea	{02}{012}{01}1{123}{12}{1234}{012346}00{0123}{01}{01}{12}1{01}{01}{01}{15789}101{01}2000100{01}

Appendix 5. Continued.

Democratic method

Acorus.ame	0100-00100110010--?1010100??000
Acorus.cal	0100-00100110010--?1010100??000
Amborella	010?1??100??00111021O1?02000000
Anethum	021112121111O010--5101000000000
Arabidopsis	02111312101100l0?05101010000000
Atropa	021111121011001110410101O000000
Brassica	02111312101100l0?05101010000000
Buxus	00111113200000l0?05101010000000
Calycanthus	00111110101100111051010110000000
Chloranthus	02?11?11?0110010--5101010000000
Citrus	021111121111011030510l0?00??000
Coffea	02111112111l0?102051010?00??000
Cucumis	02111102111l0?1011510l0??0??000
Cycas	0100-01100100010--3001010000000
Daucus	021112121111O010--5101000000000
Dioscorea	0100-01100110010104101020000000
Drimys	0101011100100?10--?101010000000
Elaeis	0100001100100O1?105101???0??000
Eucalyptus	021112021111021000010100000000O
Ginkgo	010?101100?0001?10?00101000?000
Glycine	02111112111100101061000?0000000
Gossypium	00111123201l?2111111010?0000000
Helianthus	02111112111100111141?10?0000000
Hordeum	011111110000121100510102000l000
Illicium	0111011?10??00112051010000000O
Ipomoea	02111112101100111041010l0000000
Jasminum	02111112101100101051010?00??000
Lactuca	02111112111100111141?10?0000000
Liriodendron	0100102100110O1?00510101O0000000
Lotus	02111112111100101061000?0000000
Manihot	02111112111100101051010l?000000
Medicago	02111112111100101061000?0000000
Morus	02111112111102101061010?0000000
Musa	00111120?00?000110--------??000
Nandina	02111112101100111051010l0000000?
Nicotiana.syl	02111112101100111041010l0000000
Nicotiana.tab	02111112101100111041010l0000000
Nicotiana.tom	02111112101100111041010l0000000
Nuphar	010?10?10011?0111??10101?000000
Nymphaea	010?10?10011?0111??10101?000000
Oenothera	02111202111102100001010000000O
Oryza	011111110000121100510102000l000
Panax	021112121111O010--5101000000000
Passiflora	02111112111100101051010l?000000
Pelargonium	02111122101100111051010?0001000
Phalaenopsis	01011111001100111051000?0000000
Pinus	011111110000001020?001010000000
Piper	01011101001100l001?10101O0??000
Platanus	02110212100100101051010000000O
Populus	02111112111100101051010l?000000
Ranunculus	02111112101100111051010l0000000?
Saccharum	011111110000121100510102000l000
Scaevola	02111112111100111141?10?0000000
Solanum.bul	02111112101100111041010l0000000
Solanum.lyc	02111112101100111041010l0000000
Solanum.tub	02111112101100111041010l0000000
Sorghum	011111110000121100510102000l000
Spinacia	02111112101100111041010??00?000

Appendix 5. Continued.

Trachelium	021111121111001111141?10?0000000
Triticum	011111110000121100510102000100 0
Typha	011111110000121100510102000100 0
Vitis	021112121111011 0--51010??000000
Yucca	010111110011001110510 00?0000000
Zea	011111110000121100510102000100 0
Exemplar species method	
Acorus.ame	0100-01100110010--610111 00??000
Acorus.cal	0100-01100110010--510111 00??000
Amborella	010{01}1{01}{12}{01}00{01}{01}0011103101{01}02000000
Anethum	0211{23}223111?0110--910????0??000
Arabidopsis	021113{12}310100010--7101110000000
Atropa	02111123111103110 0{79}10????0??000
Brassica	0211111310110010--71010--000000
Buxus	00111126200000 1{01}007101110000000
Calycanthus	020111221011 0010--7101111000000
Chloranthus	02111{13}1410110010--7101110000000
Citrus	021111241111011 0--71011?00??000
Coffea	0{12}11{12}12{34}{12}1{12}1001120{6479}1?????0??000
Cucumis	02110{12}3310010310--710????0??000
Cycas	0100-02100100010--4001110000000
Daucus	021122231111?0110--{89}10110000000{01}
Dioscorea	0100-03210110010--6101120000000
Drimys	11110{12}2100000210--7101110000000
Elaeis	0100-02100100010--7101???0??000
Eucalyptus	021102{12}31130021{01}00{068}101100000000
Ginkgo	010{01}102100{01}0001{01}10{89}00111000?000
Glycine	021111131110 0010--71???????000
Gossypium	001111{34}62001{01}{12}1111{67}10110000?000
Helianthus	02111123111100111161111110110000
Hordeum	0111112100001211 10{18}1011??0??000
Illicium	0211{01}123103000112071011 00000000
Ipomoea	001111{34}6200100111 1010?10000000{01}
Jasminum	021111{23}311110010--710????0??000
Lactuca	02111223111000 1111{67}1111?0000100
Liriodendron	0100--031001?001100{68}101110000000
Lotus	021121131110 0010--81001?00??000
Manihot	001111{34}6200?00112021011 1000000
Medicago	0211232{34}111?0010--{48}10????0??000
Morus	0211111{234}100112111081011??000000
Musa	00111140------00--0------0-?000
Nandina	021123231011 0010--{67}101110000001
Nicotiana.syl	02111123111 0010--{7A}10????000000
Nicotiana.tab	0211112{34}11110010--{7A}10????000000
Nicotiana.tom	0211112{34}11110010--{7A}10????000000
Nuphar	010{01}10{23}1001110111{01}?20111?00?000
Nymphaea	010{01}102110111011{012}1?20111?00?000
Oenothera	0211{01}24310010311({02}00100100000010
Oryza	0111112100001211 1011010--0??00{01}
Panax	02111{12}23111?0{01}10--{6A}1011?000?000
Passiflora	021111125103?0010--7101110000000
Pelargonium	02112123101{01}0010--7101{01}--001000
Phalaenopsis	20?11100------10--01001?0000000
Pinus	0100-03100100010--7001110000000
Piper	0101111100110011{01}18{12}011100??000
Platanus	021111{12}310110010--7101100000000
Populus	00111120------1120710111 0000000
Ranunculus	0211112{345}10110011106101 1?20??000

Appendix 5. Continued.

Saccharum	011111121000?12110071010--0??000
Scaevola	021121231111021110{06}1111?00??000
Solanum.bul	021112231111031120010111?0??001
Solanum.lyc	021112231111031120610111?0??001
Solanum.tub	021112231111031120610111?0??001
Sorghum	011111121000?12110011010--001000
Spinacia	001111262001001110610???0??000
Trachelium	021111231100?10111041011110000000
Triticum	011111{23}1000012111011011--001000
Typha	111111{123}1000{01}0110--71010--00?000
Vitis	0211{12}21{34}11110110--{78}1011??000000
Yucca	0100-0{23}100110010--610???000000
Zea	011111{34}10000121110710112000100{01}
Exemplar species method, polymorphic data points treated as missing data	
Acorus.ame	0100-01100110010--610111100??000
Acorus.cal	0100-01100110010--510111100??000
Amborella	010?1???00??00111031012?02000000
Anethum	0211?223111?0110--910????0??000
Arabidopsis	021113?310100010--7101110000000
Atropa	0211112311110311002?10????0??000
Brassica	021111131011001O--71010--000000
Buxus	0011112620000012?007101110000000
Calycanthus	020111221011001O--7101111000000
Chloranthus	02111?1410110010--7101110000000
Citrus	021111241111011O--71011?00??000
Coffea	0?11?12??1?1001120?1?????0??000
Cucumis	02110?3310010310--710????0??000
Cycas	0100-02100100010--40011100000000
Daucus	021122231112?0110--?10110000000?
Dioscorea	0100-03210110010--61011200000000
Drimys	11110?2100000210--7101110000000
Elaeis	0100-02100100010--7101???0??000
Eucalyptus	021102?31120021?00?1011?0000000
Ginkgo	010?102100?0001?10?00111000?000
Glycine	021111131110001O--71????????000
Gossypium	001111?62001??1111?10110000?000
Helianthus	021111231111001111161111110110000
Hordeum	011111210000121110?1011??0??000
Illicium	0211?123102000112071011000000000
Ipomoea	001111?62001001111010?100000000?
Jasminum	021111?311110010--710????0??000
Lactuca	021112231110001111?1111?0000100
Liriodendron	0100--031001?001100?101110000000
Lotus	02112113111000l0--81001?00??000
Manihot	001111?6200?001120210111l000000
Medicago	0211232?111?0010--?10????0??000
Morus	0211111?100112111081011??000000
Musa	00111140------00--0------0-?000
Nandina	021123231011001O--?101110000001
Nicotiana.syl	02111123111100l0--?10????000000
Nicotiana.tab	0211112?11110010--?10????000000
Nicotiana.tom	0211112?11110010--?10????000000
Nuphar	010?10?100111011l??20111?00?000
Nymphaea	010?102110111011?1?20111?00?000
Oenothera	0211?2431001031l?001001000000l0
Oryza	011111210000121110l1010--0??00?
Panax	02111?23111?0?10--?1011?000?000
Passiflora	021111121111001010510101?000000
Pelargonium	02112123101?0010--7101?--001000

Appendix 5. Continued.

Phalaenopsis	20?11100------10--01001?00000000
Pinus	0100-03100100010--70011100000000
Piper	0101111100110011?18?011100??000
Platanus	021111?310110010--71011000000000
Populus	00111120------1120710111000000
Ranunculus	0211112?10110011106101l?20??000
Saccharum	011111210000?12110071010--0??000
Scaevola	021121231111021110?1111?00??000
Solanum.bul	0211122311110311200l0111?0??001
Solanum.lyc	021112231111031120610111?0??001
Solanum.tub	021112231111031120610111?0??001
Sorghum	011111210000?12110011010--001000
Spinacia	00111126200100111061O????0??000
Trachelium	021111231OO?10111041011100000000
Triticum	011111?1000012111011011--001000
Typha	111111?1000?0110--71010--00?000
Vitis	0211?21?11110110--?1011??000000
Yucca	0100-0?100110010--610????000000
Zea	011111?10000121110710112000100?

Appendix 6. Voucher details for pollen grains shown in Figure 3.

Taxon	Collection	Locality	Herbarium	Figure
Acacia nilotica (L.) Willd. ex Delile (Fabaceae)	*J. B. Allen 229*	Afgoi, Somalia	E	4C
Acorus gramineus Sol. ex Aiton (Acoraceae)	*K. L. Chu 2272*	Tien Chuan Hsien, China	E	4E
Adenocaulon chilense Less. (Asteraceae)	*T. M. Pedersen 1494*	Los Lagos, Argentina	US	4R
Aesculus hippocastanum L. (Sapindaceae)	—	cult. nr. Haywards Heath, United Kingdom	—	4P
Alfredia cernua (L.) Cass. (Asteraceae)	*Roginskaya 74*	Russia	K	4X
Cabobanthus bullulatus (S. Moore) H. Rob. (Asteraceae)	*Lisowski 357*	Haut-Katanga, Democratic Republic of Congo	K	4I
Centrapalus pauciflorus (Willd.) H. Rob. (Asteraceae)	*N. Hepper 3760*	Kati, Mali	K	4G
Crepis napifera (Franch.) Babc. (Asteraceae)	*G. Forrest 2987*	Yunnan, China	BM	4D
Croton argyratus Blume (Euphorbiaceae)	*B. S. Expedition 201*	Thailand	E	4U, V
Cullumia rigida DC. (Asteraceae)	*T. Trinder-Smith 182*	South Africa	US	4T
Dampiera stricta (Sm.) R. Br. (Goodeniaceae)	*W. E. Fisher 99*	New South Wales, Australia	E	4W
Dioscorea nipponica Makino (Dioscoreaceae)	*O. Zhurban 6856*	Primorje, Russia	E	4F
Dioscorea pyrenaica Bubani & Bordere ex Gren. (Dioscoreaceae)	*C. Packe s.n.*	Spanish Pyrenees, Spain	E	4Q
Illicium floridanum J. Ellis (Illiciaceae)	*F. H. Utech 83-039*	Louisiana, U.S.A.	KUN	4O
Lathyrus pratensis L. (Fabaceae)	*Th. Constantinidis 8046*	Mount Kratsovon, Thessalia, Greece	E	4S
Nouelia insignis Franch. (Asteraceae)	*J. Rock 11714*	Yunnan, China	US	4J
Oldenburgia paradoxa Less. (Asteraceae)	*Owan 374*	South Africa	US	4L
Plantago psyllium L. (Plantaginaceae)	*Davis 52297*	Algeria	E	4A
Poa bulbosa L. (Poaceae)	*D. Davis 63473*	Agrigento, Sicily, Italy	E	4M
Rhododendron wallichii Hook. f. (Ericaceae)	*R. C. Poudel 10*	Royal Botanic Garden Edinburgh	E	4B
Scorzonera hispanica L. (Asteraceae)	—	cult. Chelsea Physic Garden, United Kingdom	—	4N
Tragopogon longifolius Heldr. & Sartori (Asteraceae)	*F. Guiol 1007*	Attica, Greece	BM	4K
Ulmus glabra Huds. (Ulmaceae)	*Culte 15278*	Royal Botanic Garden Edinburgh	E	4H

EVOLUTION OF ANGIOSPERM POLLEN. 2. THE BASAL ANGIOSPERMS[1]

Lu Lu,[2,3] *Alexandra H. Wortley,*[4] *De-zhu Li,*[2,3] *Hong Wang,*[2,3*] *and Stephen Blackmore*[4]

Abstract

Recently, evolutionary relationships among the five major lineages of the basal angiosperms, i.e., Amborellanae, Nymphaeanae, Austrobaileyanae, Magnolianae, and Ceratophyllanae, still remain incompletely or controversially resolved. This study reviews and reevaluates the origin, evolution, and systematic significance of pollen morphology in basal angiosperms. Pollen grains from 34 species of 31 genera in 23 families of the basal angiosperms are investigated, using light microscopy (LM) and scanning electron microscopy (SEM), to illustrate the pollen diversity across the grade. Pollen data for 57 genera, representing all nine orders and 28 families of the basal angiosperms, were obtained from previous work and new investigations and were used to optimize character states onto recent molecular phylogenetic estimates. Two 18-character datasets were generated: a comprehensively coded dataset optimized with Fitch parsimony onto 12 recent phylogenies with differing topologies to evaluate the degree of pollen systematic significance of each topology, and a democratically coded dataset optimized by using Fitch parsimony, maximum likelihood, and hierarchical Bayesian inference onto a single recent phylogeny based on molecular data from Soltis et al. to evaluate the disparity of pollen evolutionary patterns among the three methods. Pollen morphology of the basal angiosperms is highly diverse, particularly in terms of pollen size, aperture number, aperture position, ectoaperture shape, tectum sculpture, and infratectal structure. Based on both datasets, the plesiomorphic pollen type for angiosperms was inferred to comprise monads of heteropolar, spheroidal, mono-apertural grains, with distal, colpate apertures, sculptured aperture membranes, and an infratectum and foot layer. Of the basal angiosperm phylogenies considered, when taxon sampling is taken into account, pollen characters provide greatest support for the topology represented in the three-mitochondrial-gene study from Qiu et al., in terms of number of synapomorphies plus what we term "likely synapomorphies" in which the subtending node is ambiguous and, therefore, the exact position of the synapomorphy is ambiguous. Thirty-six various lineages above family level are supported by pollen morphological synapomorphies or likely synapomorphies. We discuss the systematic significance of pollen morphology in basal angiosperm clades including the ANITA group (Amborellaceae, Nymphaeaceae, Schisandraceae, Trimeniaceae, and Austrobaileyaceae), Ceratophyllales, Chloranthales, and magnoliids, as well as in related lineages, i.e., monocots and basal eudicots. We also discuss present limitations in inferring the evolutionary history of pollen morphology in basal angiosperms.

Key words: Amborellales, ancestral character reconstruction, Austrobaileyales, basal angiosperms, Ceratophyllales, character evolution, Chloranthales, magnoliids, Nymphaeales, pollen morphology, systematic significance.

The basal angiosperms are defined as those species at the tips of the earliest diverging flowering plant lineages (i.e., all angiosperms with the exception of monocots and eudicots) and comprise a grade of separate evolutionary lineages, together containing a few hundred species. The nature of the basal angiosperms is intrinsically linked with the pattern, process, and timing of the origin of the angiosperms and is perhaps the most controversial topic in plant evolution, one that has generated an immense literature. Over the last 20 years, since the angiosperms have unequivocally been determined as a monophyletic

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group, they have been subject to the most intensive phylogenetic analyses ever conducted on any group of taxa, using a variety of methods and different combinations of molecular markers for clarifying relationships, particularly among extant basal angiosperms (reviewed by Qiu et al., 2006; Hansen et al., 2007; Jansen et al., 2007; Zavada, 2007; Soltis et al., 2007, 2008; Endress & Doyle, 2009).

An initial proposal of *Kadsura* Juss. (Schisandraceae) as the most primitive angiosperm by Delpino (1890) was refuted by recent studies, starting with Chase et al. (1993), whose analysis showed *Ceratophyllum* L. to be the basalmost extant angiosperm based on a large *rbc*L tree of seed plants. However, combined-gene analyses over the past decade or more have now confirmed, instead, the ANITA grouping (Amborellaceae, Nymphaeaceae, Schisandraceae, Trimeniaceae, and Austrobaileyaceae) as the basalmost grade of angiosperms (Mathews & Donoghue, 1999; Parkinson et al., 1999; Qiu et al., 1999; Soltis et al., 1999; Barkman et al., 2000; Graham & Olmstead, 2000). The relationships of the other earliest branching lineages, including magnoliids, Ceratophyllaceae, Chloranthaceae, monocots, and basal eudicots, have gradually been resolved with increasing confidence through a series of phylogenetic investigations (e.g., Chase et al., 1993; Qiu et al., 1999, 2000, 2005, 2006; Graham & Olmstead, 2000; Savolainen et al., 2000; Soltis et al., 2000, 2007, 2008; Zanis et al., 2002; Aoki et al., 2004; Hansen et al., 2007; Jansen et al., 2007; Moore et al., 2007; Endress & Doyle, 2009). By the time of the most recent Angiosperm Phylogeny Group classification (Angiosperm Phylogeny Group, 2009), the basal angiosperms were mainly agreed to comprise the five successively evolved major clades identified as the Amborellanae (Amborellales), the Nymphaeanae (Nymphaeales), the Austrobaileyanae (Austrobaileyales), the Magnolianae (Piperales, Canellales, Magnoliales, Laurales, and Chloranthales), and the Ceratophyllanae (Ceratophyllales).

Although these monophyletic clades within basal angiosperms have each been firmly ascertained from both molecular and morphological data, their systematic relationships remain incompletely resolved and controversially supported, depending on the genomes and reconstruction approaches used (Hansen et al., 2007; Moore et al., 2007). It will not be easy to determine whether Amborellanae alone is the first-branching angiosperm, or whether it groups with Nymphaeanae as a first branch (Qiu et al., 2000, 2006; Leebens-Mack et al., 2005; Jansen et al., 2007). No recent analyses have found consistent support for their relationships (reviewed by Hansen et al., 2007; Moore et al., 2007). The placements of the Ceratophyllales and Chloranthales in particular are highly unstable at present (reviewed by Qiu et al., 2006; Endress & Doyle, 2009). Thus, understanding the origin and early relationships of the angiosperms remains a formidable challenge.

Collating intensive morphological evidence can facilitate the understanding of these evolutionary uncertainties, which may not be resolved by molecular analyses in the short term. Although morphological investigations may not themselves solve the current impasse, it is useful to consider possible morphological support for alternative molecular tree topologies (Renner et al., 2000). Valuable morphological characters with systematic significance, such as floral morphology, ontogeny, and breeding systems, have been elucidated in detail for basal angiosperms over past decades (Donoghue & Doyle, 1989; Doyle et al., 1994; Doyle, 1996; Doyle & Endress, 2000; Floyd & Friedman, 2001; Endress, 2010, 2011). Palynology is considered a unique source of morphological information in that no other dataset can provide so great an amount of information from so little material in so short a time (Walker & Doyle, 1975). In previous studies, pollen characters provided insights into aspects of taxonomy and phylogenetics for many taxa within basal angiosperms, including the Winteraceae (Sampson, 1993, 2007), Calycanthaceae, Siparunaceae and Atherospermataceae (Renner, 1999), *Maloutchia* Warb. (Sauquet et al., 2003), Nymphaeaceae (Podoplelova & Ryzhakov, 2005), Annonaceae (Doyle & Le Thomas, 1997), Monimiaceae (Renner, 1998), and Schisandraceae (Wang et al., 2010).

The evolution of pollen morphology in the basal angiosperms has been debated for a long time (Muller, 1970; Walker, 1974, 1976; Walker & Doyle, 1975; Walker & Walker, 1984; Doyle & Hotton, 1991; Gabarayeva, 1991; Brenner, 1996; Friis et al., 1999, 2000; Sampson, 2000a, 2007; Furness & Rudall, 2004; Doyle, 2005; Zavada, 2007). There have been considerable controversies as to the nature of the most primitive angiosperm pollen grain and evolutionary trends (Walker & Doyle, 1975; Walker & Walker, 1984; Brenner, 1996; Doyle, 2005). Due to the continuing uncertainty in phylogenetic estimates, and incomplete or unavailable palynological data for certain extant and fossil taxa, the identification of plesiomorphic palynological characters, and related transitions in pollen morphological evolution, remain difficult. Nevertheless, our increasingly robust understanding of angiosperm phylogeny allows a more secure reconstruction of the morphological characters of extant angiosperms and their early

evolution than ever before (Endress & Doyle, 2009; Wortley et al., 2015).

In this paper, we investigate the diversity and evolution of pollen in basal angiosperms and related taxa using a combination of reanalyzed, previously published information and new data. This is set in the context of recent molecular phylogenetic hypotheses and follows the protocol outlined in Wortley et al. (2015). First, we optimize the distribution of selected pollen characters onto alternative molecular-based phylogenetic estimates from recent studies to infer plesiomorphic and synapomorphic (or likely synapomorphic) states for palynological characters. This enables us to interpret evolutionary patterns in terms of the inferred plesiomorphic pollen characters of angiosperms, and of the systematic value of palynological characters to elucidate some of the uncertain relationships among the early diverging lineages in divergent tree topologies. Second, we optimize the states of selected pollen characters onto a maximum likelihood tree generated from the molecular data of Soltis et al. (2011) using three methods, i.e., Fitch parsimony, maximum likelihood, and hierarchical Bayesian inference, to test the differences in inferred patterns of state change and the significance of disagreements among these methods. Finally, we discuss the constraints facing current research into plesiomorphic palynological characters and their evolution in basal angiosperm lineages.

Materials and Methods

Study taxa

For new palynological observations, pollen grains from 34 species of 31 genera in 23 families of basal angiosperms were observed with light microscopy (LM) and scanning electron microscopy (SEM). Pollen samples were taken from the living collections at Kunming Botanic Garden (KBG) and Xishuangbanna Tropical Botanical Garden (HITBG), and the herbaria of the Kunming Institute of Botany, China (KUN), Royal Botanic Garden Edinburgh, U.K. (E), and the Australian National Herbarium, Australia (CANB) (cf. Appendix 1). Preparation of pollen grains for observation followed the acetolysis method of Erdtman (1960). To observe the stratification of the pollen exine, pollen grains used for LM examination were embedded in glycerine jelly on glass slides. Pollen size (the maxium diameter of a single grain) was measured under LM. To prepare specimens for SEM examination, acetolyzed grains were suspended in 95% ethanol for dehydration, then mounted on specimen stubs, and sputter-coated with gold-palladium. Pollen morphology (Figs. 1–6) was examined using a Hitachi (Tokyo, Japan) S-4800 SEM at 10 KV (KUN) and a Zeiss (Oberkochen, Germany) DSM 962 SEM at 15 KV (E). Image editing was accomplished using Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, California, U.S.A.).

Taxon sampling and choice of base phylogenies

We used 12 base tree topologies from recent molecular phylogenies focusing on the basal angiosperms (Qiu et al., 1999, 2000, 2006; Graham & Olmstead, 2000; Mathews & Donoghue, 2000; Soltis et al., 2000, 2005, 2007, 2011; Müller et al., 2006; Jansen et al., 2007; Moore et al., 2007), which together represent all conflicting topologies found in the recent literature (Figs. 7–9). One topology was a modified combination of Jansen et al. (2007) and Moore et al. (2007), giving a topology similar to that of Soltis et al. (2011) but with different taxon sampling. Two topologies were taken from Qiu et al. (2006), i.e., one consensus tree of likelihood and parsimony analyses of eight genes, and one consensus tree of likelihood analyses of three mitochondrial genes.

All of these trees were modified by reducing the number of terminals, in particular within basal eudicots and monocots, which were not the focus of this study. All trees used gymnosperms as outgroups, with the exception of that from Mathews and Donoghue (2000), which was rooted without outgroups. The total number of terminals on all trees varied from 16 to 78, featuring 57 exemplar genera representing all nine orders and 28 families of the basal angiosperms, representing the breadth of morphological and palynological diversity within the group, with eight genus-level exemplars spanning four orders and eight families of the gymnosperms as outgroups, 10 genus-level exemplars spanning three orders and eight families of basal eudicots, and three genus-level exemplars spanning two orders and three families of monocots. We worked with genus-level terminals (cf. Appendix 1), and our systematic treatment follows the classification of the Angiosperm Phylogeny Group (2009) and Soltis et al. (2011).

In addition, to enable further comparative studies, a phylogenetic tree with branch lengths was generated based on molecular data of all 17 genic regions for 68 out of 640 taxa in total as utilized in Soltis et al. (2011; the most recent authoritative topology of angiosperms) that were obtained from TreeBASE (S11267, see <http://www.treebase.org>; see tree diagrams, Fig. 10). The 68 taxa were all covered within the 78 taxa described above (cf. Appendix 1), including 50 genera of basal angiosperms, seven genera of basal eudicots, three genera of monocots, and eight genera of gymnosperm as outgroups.

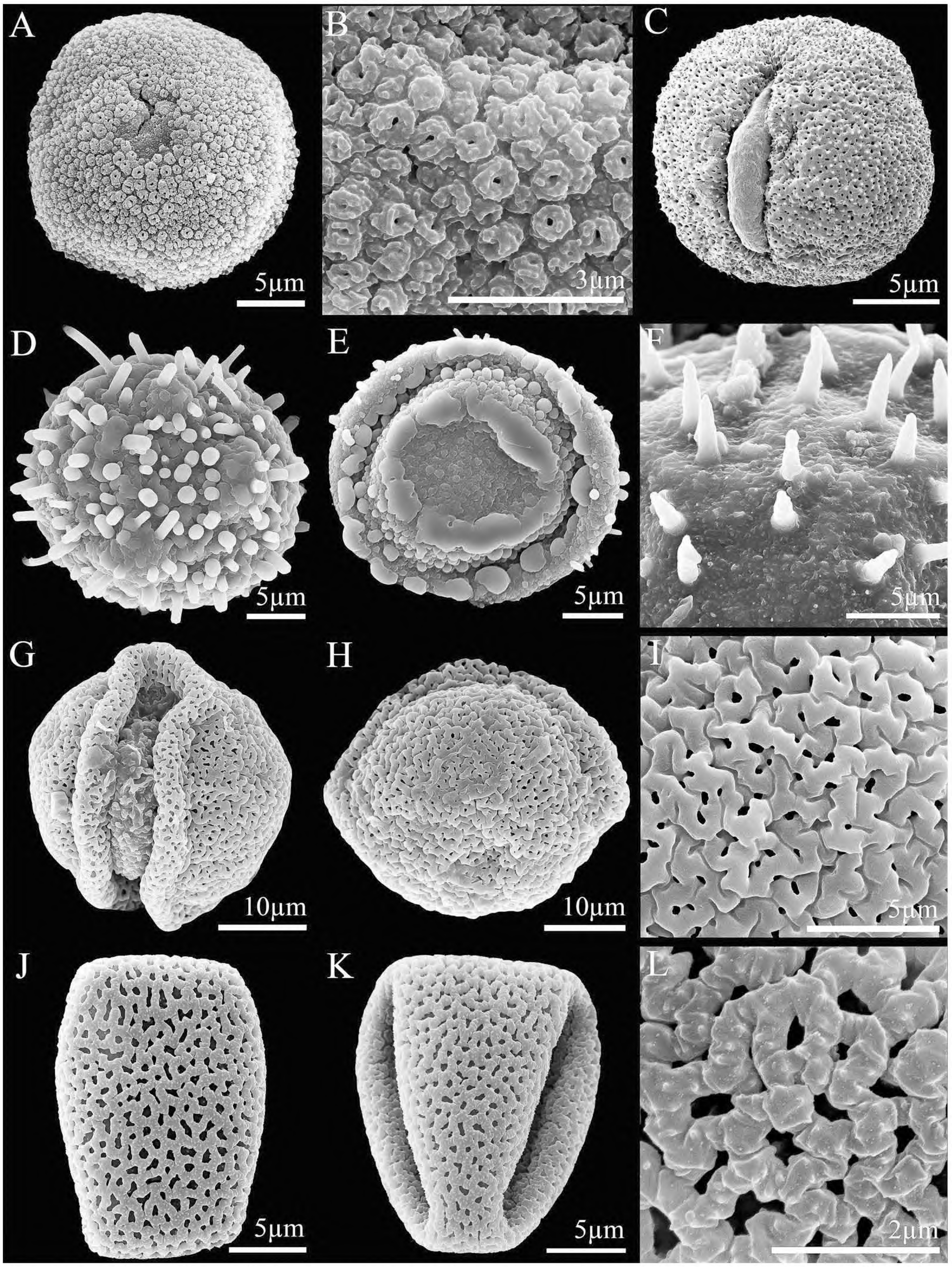


Figure 1. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). A, B. *Amborella trichopoda* Baill. —A. Distal polar view, showing monad, globose, monoporate pollen grain. —B. Detail showing *Amborella*-type tectum with spinulose supratectal elements. —C. *Trithuria australis* (Diels) D. D. Sokoloff, Remizowa, T. D. Macfarl. & Rudall, distal polar view, showing a distal colpus and perforate tectum with spinulose supratectal elements. D, E. *Nymphaea alba* L. —D. Distal polar view, showing zonate aperture located at the distal pole. —E. Proximal polar view, showing spheroidal shape, echinate and verrucate supratectal elements. —F. *Nuphar luteum* Walp., showing echinate and verrucate supratectal elements. G–I. *Austrobaileya scandens* C. T. White. —G. Distal polar view, showing heteropolarity, bilateral symmetry, and a distal colpus with sculptured aperture membrane. —H. Equatorial view, showing oblate shape. —I. Detail showing reticulate tectum with absence of supratectal elements. J–L. *Trimenia moorei* (Oliv.) Philipson. —J. Equatorial view, showing prolate shape and two colpi located at the equator. —K. Equatorial view. —L. Detail showing reticulate tectum with spinulose supratectal elements.

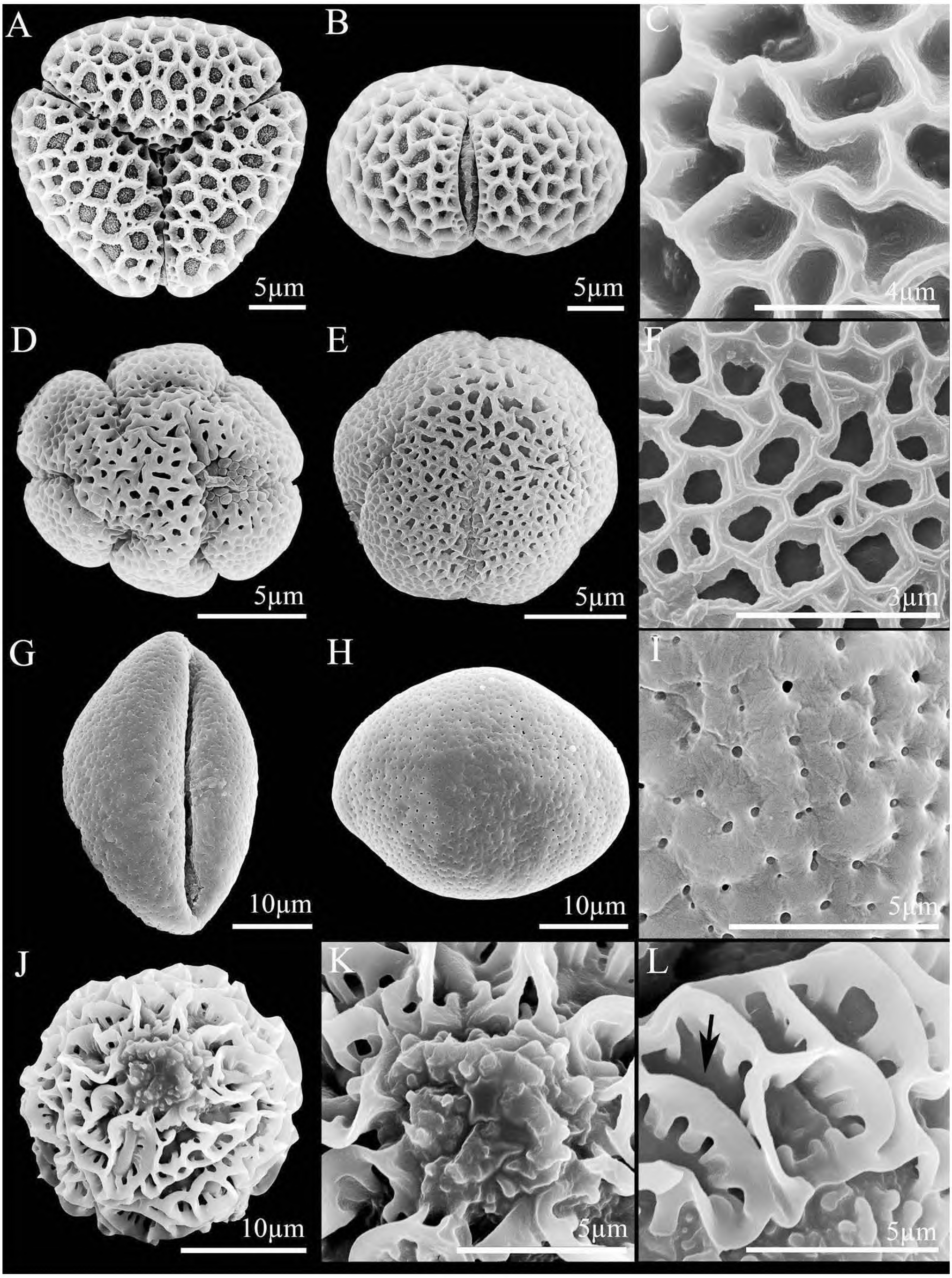


Figure 2. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). A, B. *Illicium micranthum* Dunn. —A. Polar view, showing isopolar, radially symmetrical, circular and syncolpate, triaperturate grains. —B. Equatorial view. —C. *Illicium macranthum* A. C. Sm., showing reticulate tectum. —D. *Chloranthus japonicus* Siebold, polar view, showing hexa-colpate apertures. E, F. *Chloranthus holostegius* (Hand.-Mazz.) S. J. Pei & Shan. —E. Polar view and partly equatorial view, showing hexa-colpate apertures. —F. Detail of reticulate tectum. G–I. *Canella* P. Browne, sp. indet. —G. Distal polar view, showing elliptic shape. —H. Proximal view. —I. Detail of perforate tectum. J–L. *Drimys piperita* Hook. f. —J. Tetrad. —K. Detail of porate aperture on a monad. —L. Detail of reticulate tectum, columellate infratectum; arrow indicates the foot layer.

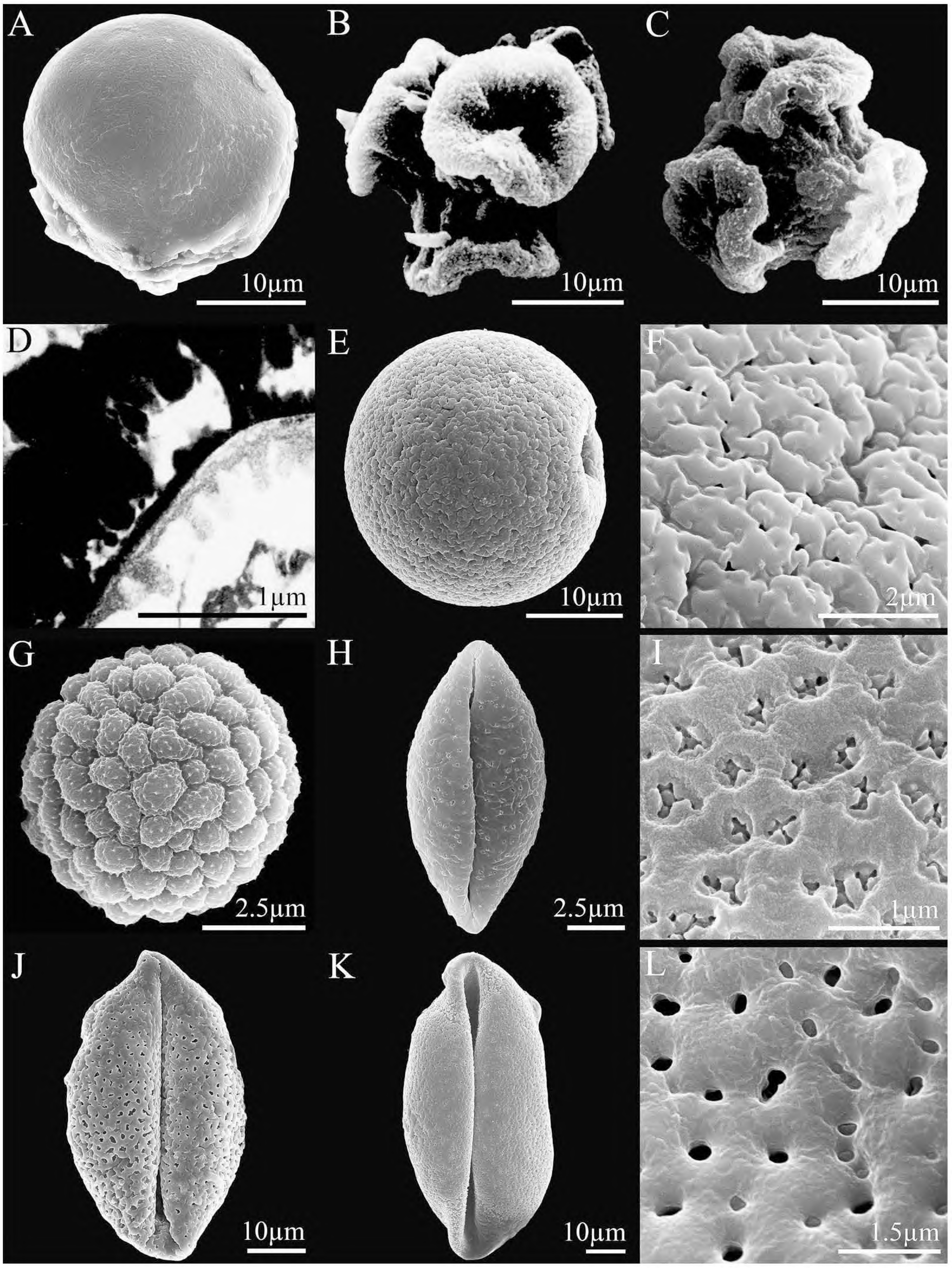


Figure 3. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). —A. *Hydnora abyssinica* A. Braun, equatorial view, showing a colpus located at the distal pole. B–D. *Lactoris fernandeziana* Phil.; images reprinted with permission from Zavada and Taylor (1986). —B, C. Tetrad. —D. TEM showing granulate infratectum. E, F. *Aristolochia contorta* Bunge. —E. Equatorial view, spheroidal pollen grain showing porate aperture. —F. Detail of rugulate tectum. —G. *Peperomia heyneana* Miq., showing apolar, radial, spheroidal, small-sized, inaperturate pollen grain and areolate tectum with spinulose supratectal elements. —H. *Saururus chinensis* (Lour.) Baill., distal polar view, showing boat-shaped pollen. —I. *Houttuynia cordata* Thunb., showing unique form of perforate tectum. —J. *Liriodendron tulipifera* L., distal polar view, showing boat-shaped pollen with large size. K, L. *Magnolia grandiflora* L. —K. Distal polar view, showing boat-shaped pollen. —L. Detail of perforate tectum.

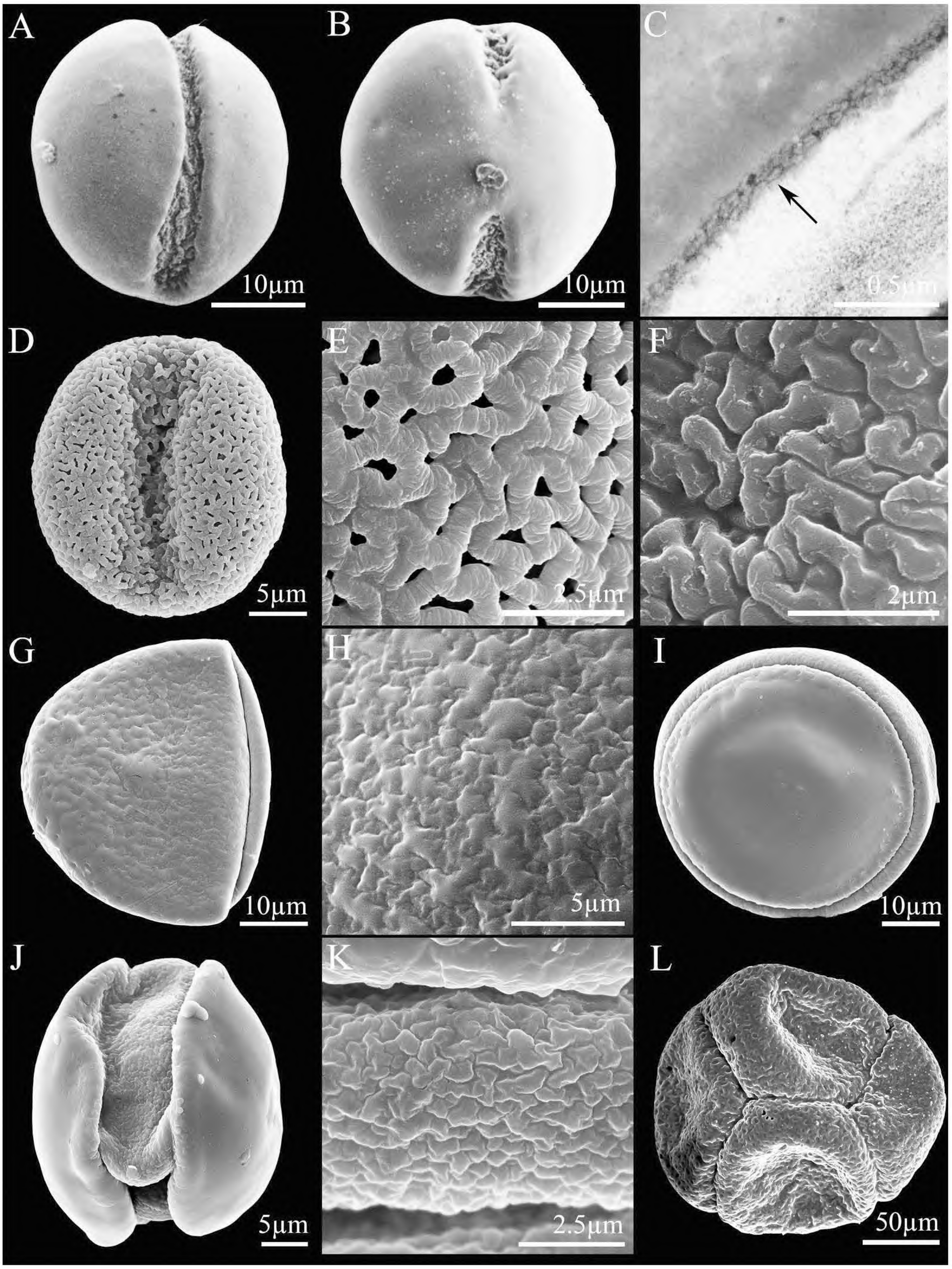


Figure 4. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). A–C. *Degeneria vitiensis* I. W. Bailey & A. C. Sm.; reprinted with permission from Sampson (2000a). —A. Distal polar view. —B. Proximal polar view. —C. TEM showing the near homogeneous nature of the exine with small granules; arrow indicates absence of a foot layer. D, E. *Knema globularia* (Lam.) Warb. —D. Distal polar view, showing subspheroidal shape, elliptic polar view, medium size, and a colpus. —E. Detail of reticulate tectum. —F. *Myristica* Gronov., sp. indet., showing rugulate tectum. G, H. *Galbulimima baccata* F. M. Bailey. —G. Equatorial view, showing a colpus located at the distal pole. —H. Detail of rugulate tectum. I–K. *Eupomatia laurina* R. Br. —I. Polar view, showing imperforate tectum. —J. Equatorial view, showing zonate aperture. —K. Detail of sculptured aperture membrane. —L. *Annona muricata* L., tetrad with very large-sized monads.

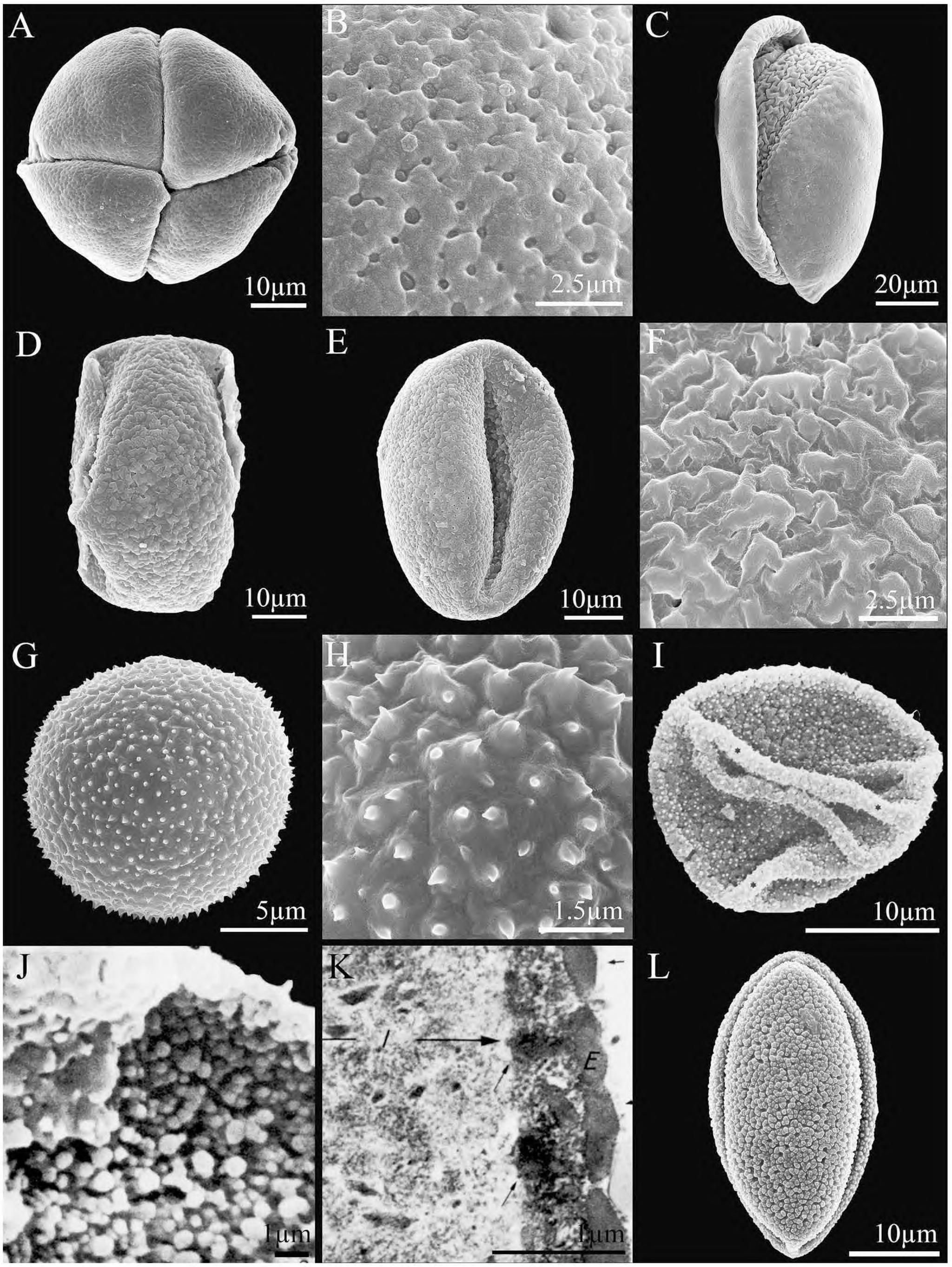


Figure 5. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). A, B. *Annona glabra* L. —A. Tetrad with medium-sized monads. —B. Detail of perforate tectum. —C. *Cananga odorata* (Lam.) Hook. f. & Thomson var. *fruticosa* (Craib) J. Sinclair, equatorial view, showing a monad with zonate aperture. D–F. *Calycanthus chinensis* (W. C. Cheng & S. Y. Chang) P. T. Li. —D. Equatorial view, showing bi-aperturate grain, apertures located at the equator. —E. Equatorial view, showing a colpus. —F. Detail of rugulate tectum. G, H. *Siparuna guianensis* Aubl. —G. Spheroidal, inaperturate pollen grain. —H. Detail of echinate supratectal elements. I–K. *Gomortega keule* (Molina) Baill., images reprinted with permission from Hesse and Kubitzki (1983). —I. Inaperturate grain. —J. TEM showing collumellate infratectum. —K. TEM showing exine structure: a channeled outer layer of intine (I) and exine (E) that is made up of tectal (short arrows) and columellar elements (longer arrows). —L. *Atherosperma moschatum* Labill., equatorial view, showing two colpi located at distal and proximal poles.

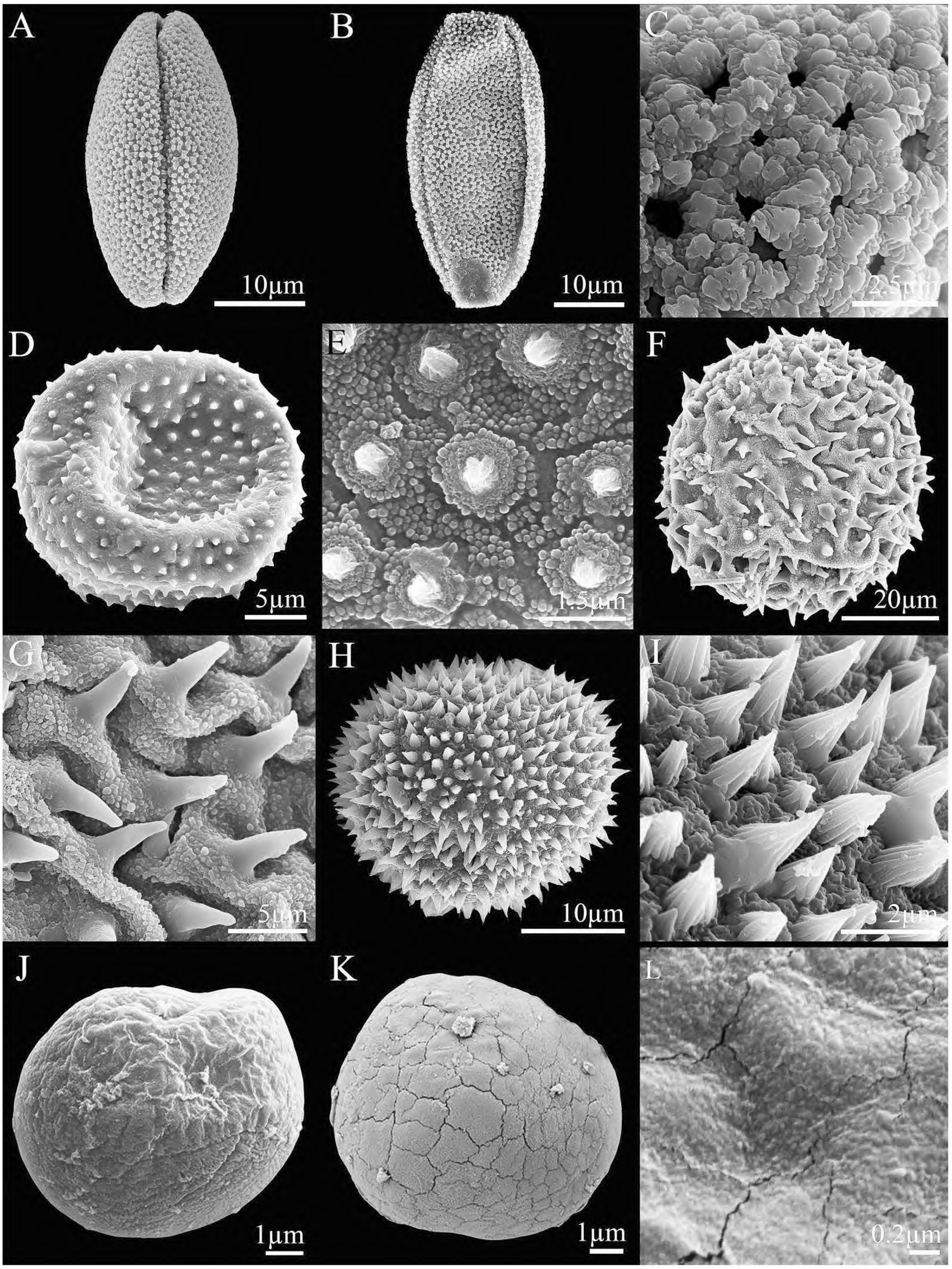


Figure 6. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). —A. *Atherosperma moschatum* Labill., polar view, showing a colpus. —B. *Doryphora sassafras* Endl., equatorial view, showing two colpi located at distal and proximal poles. —C. *Daphnandra tenuipes* G. Perkins, detail of rudimentary tectum with verrucate supratectal elements. D, E. *Laurus nobilis* L. —D. Inaperturate pollen grain. —E. Detail of echinate and verrucate supratectal elements. F, G. *Hernandia didymantha* Donn. Sm. —F. Showing spheroidal and inaperturate pollen grain. —G. Detail of echinate and verrucate supratectal elements. H, I. *Peumus boldus* Molina. —H. Showing spheroidal and inaperturate pollen grain. —I. Detail of echinate supratectal elements. J–L. *Ceratophyllum* L., sp. indet. —J, K. Spheroidal and inaperture pollen grain. —L. Detail of exine surface (exine reduced to a very thin layer).

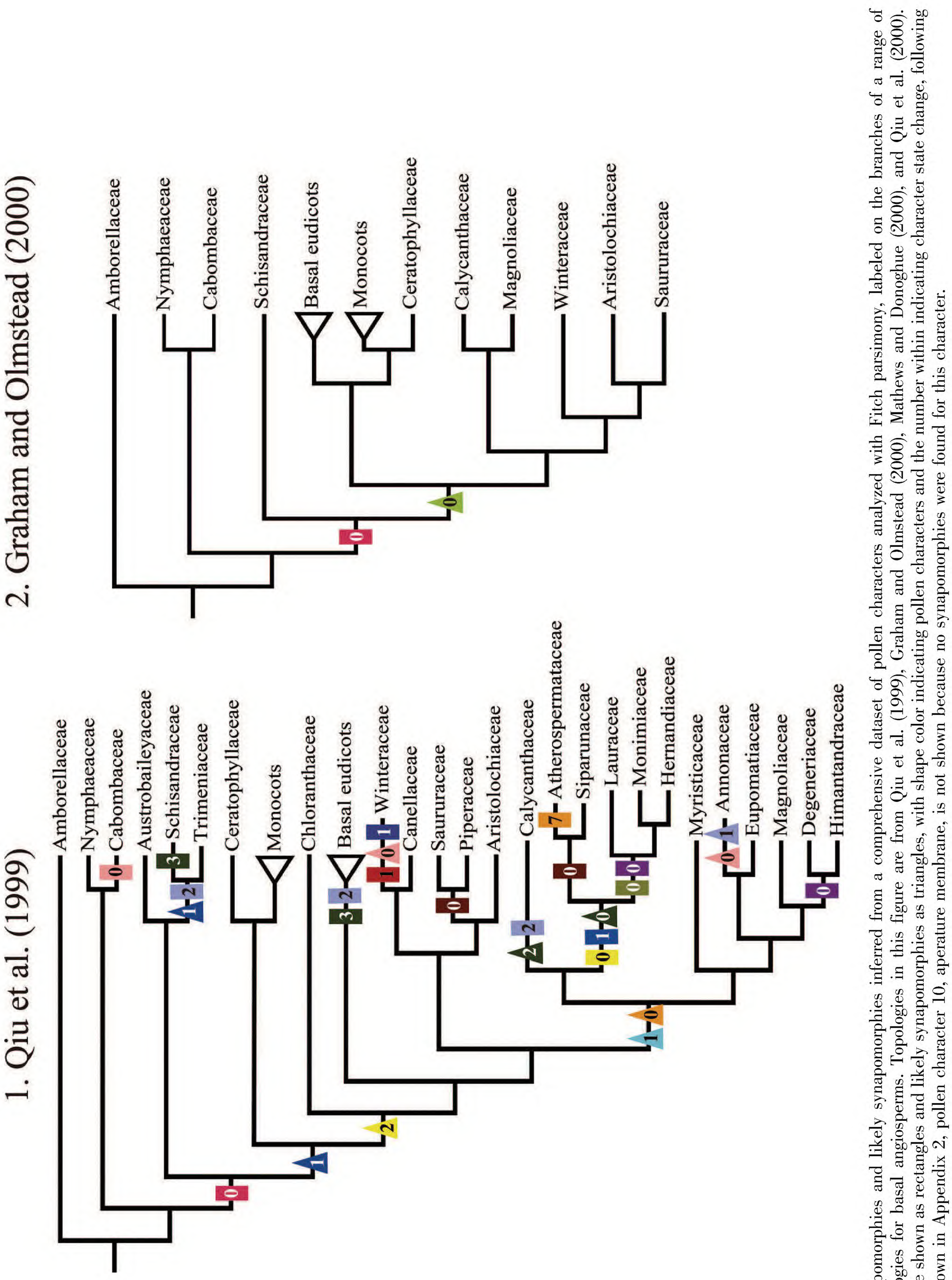


Figure 7. Synapomorphies and likely synapomorphies inferred from a comprehensive dataset of pollen characters analyzed with Fitch parsimony, labeled on the branches of a range of phylogenetic topologies for basal angiosperms. Topologies in this figure are from Qiu et al. (1999), Graham and Olmstead (2000), Mathews and Donoghue (2000), and Qiu et al. (2000). Synapomorphies are shown as rectangles and likely synapomorphies as triangles, with shape color indicating pollen characters and the number within indicating character state change, following Appendix 2. As shown in Appendix 2, pollen character 10, aperature membrane, is not shown because no synapomorphies were found for this character.

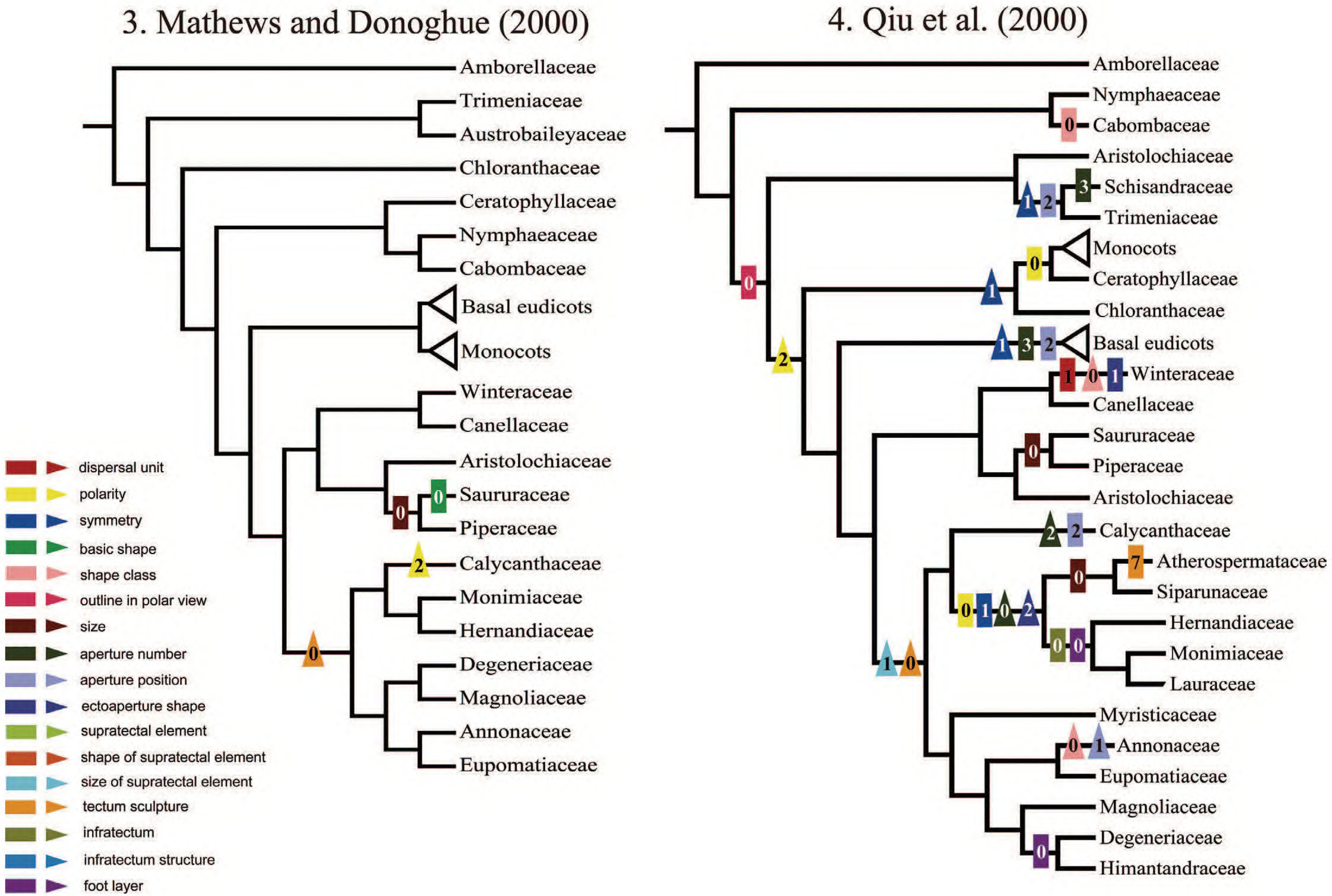


Figure 7. Continued.

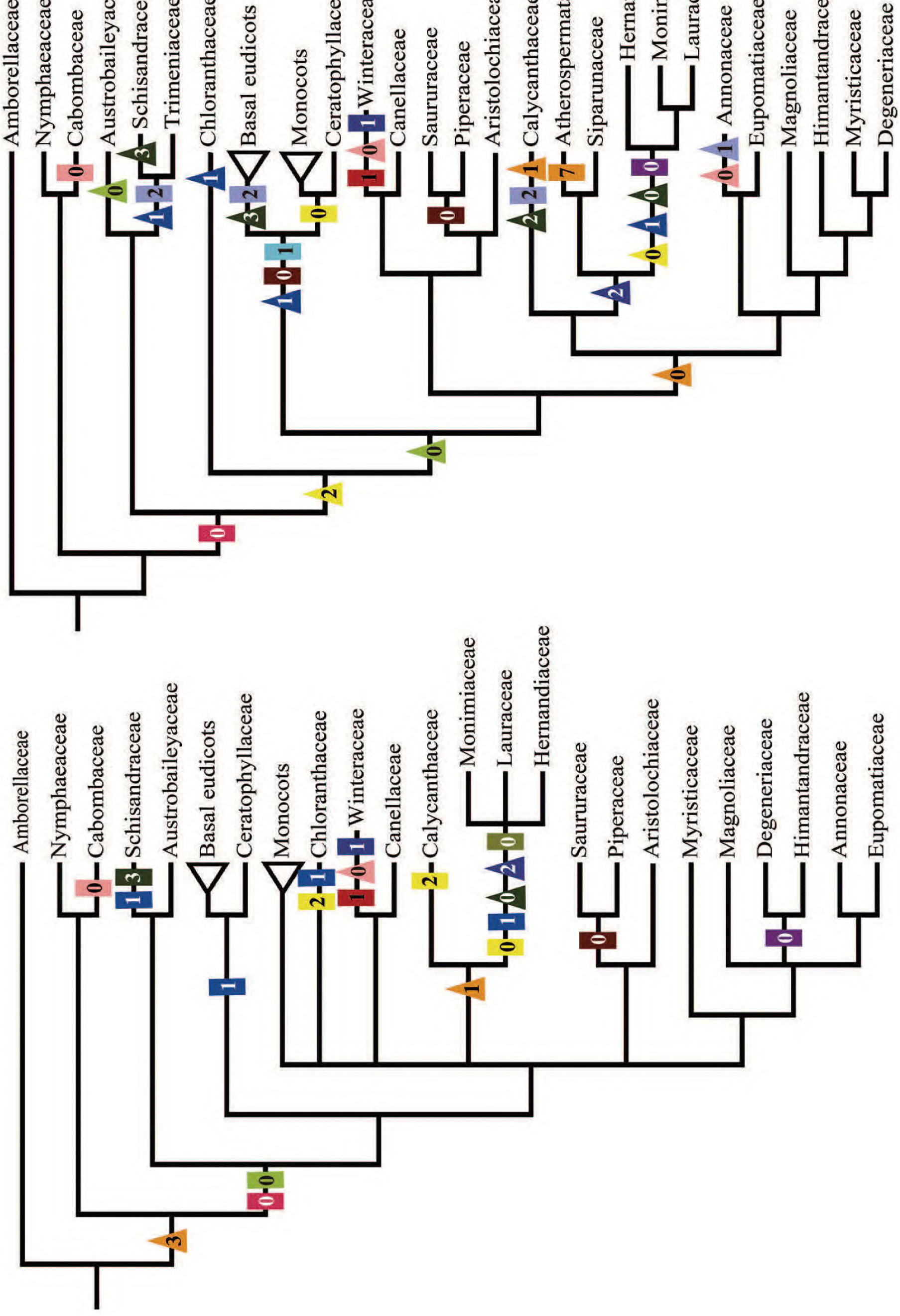


Figure 8. Synapomorphies and likely synapomorphies inferred from a comprehensive dataset of pollen characters analyzed with Fitch parsimony, labeled on the branches of a range of phylogenetic topologies for basal angiosperms. Topologies in this figure are from Soltis et al. (2000), Qiu et al. (2005), Müller et al. (2006), and Qiu et al. (2006, three genes). Synapomorphies are shown as rectangles and likely synapomorphies as triangles, with shape color indicating character state change, following Appendix 2. As shown in Appendix 2, pollen character 10, aperature membrane, is not shown because no synapomorphies were found for this character.

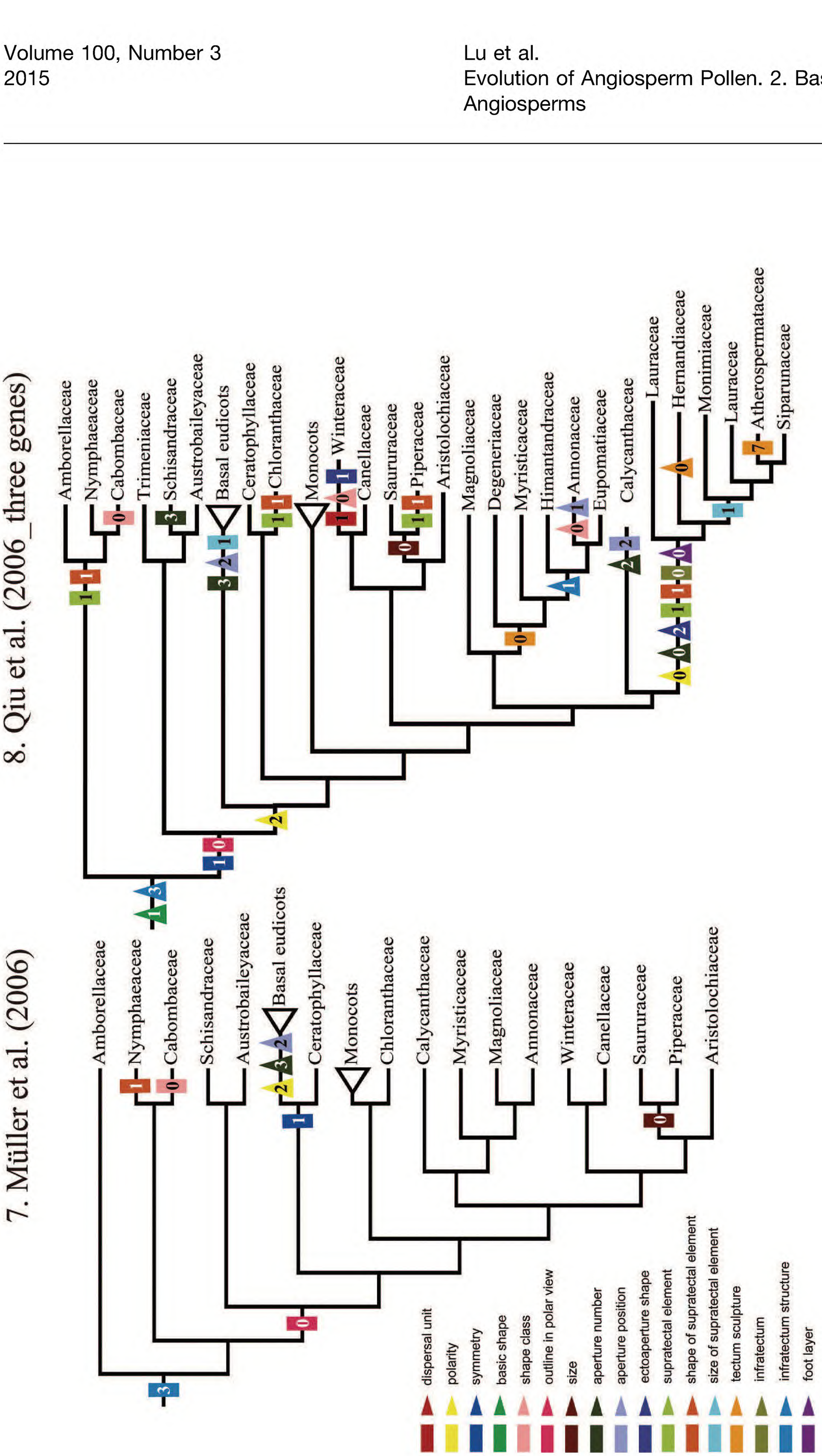
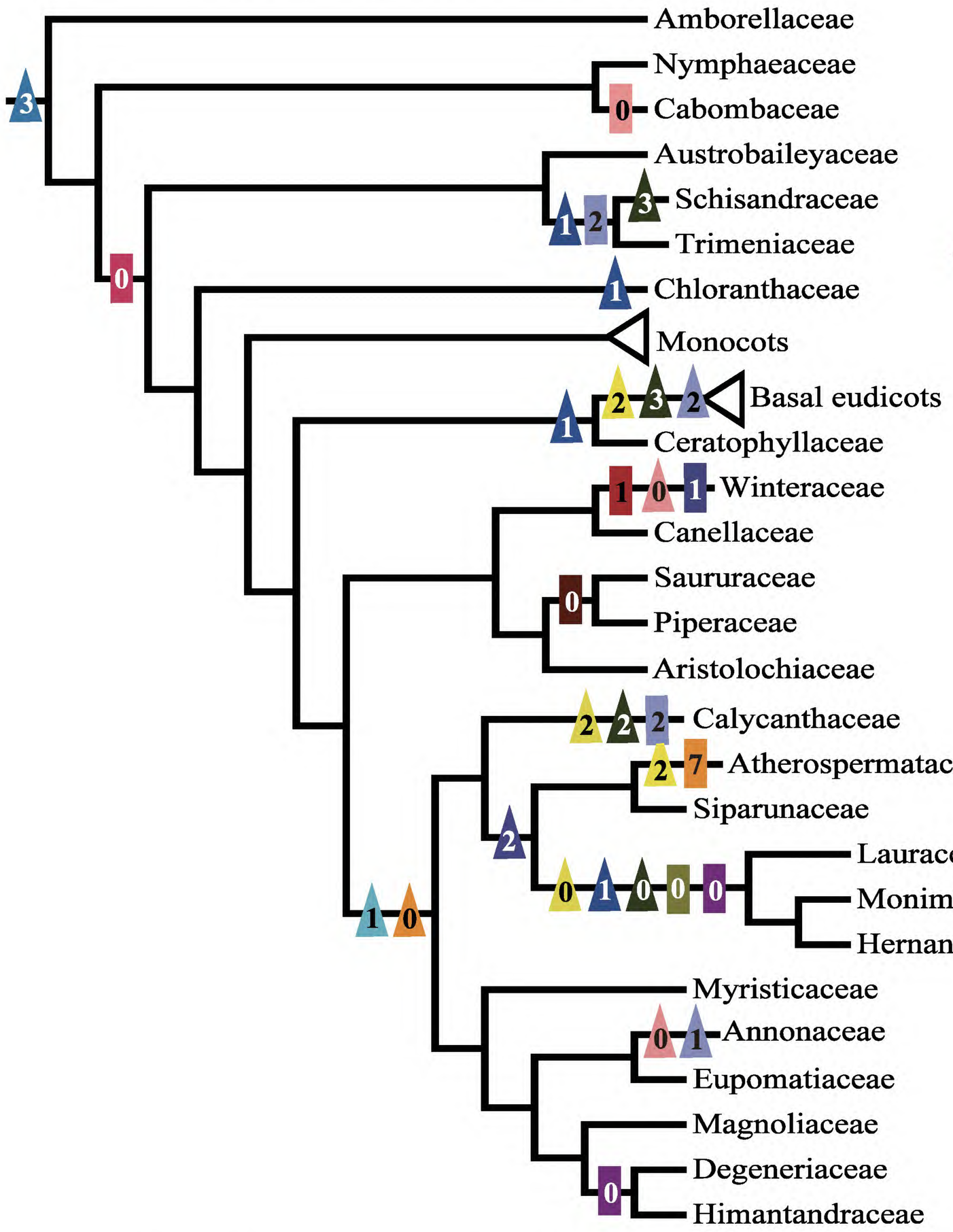


Figure 8. Continued.

9. Qiu et al. (2006_eight genes)



10. Jansen et al. (2007) & Moore et al. (2007)

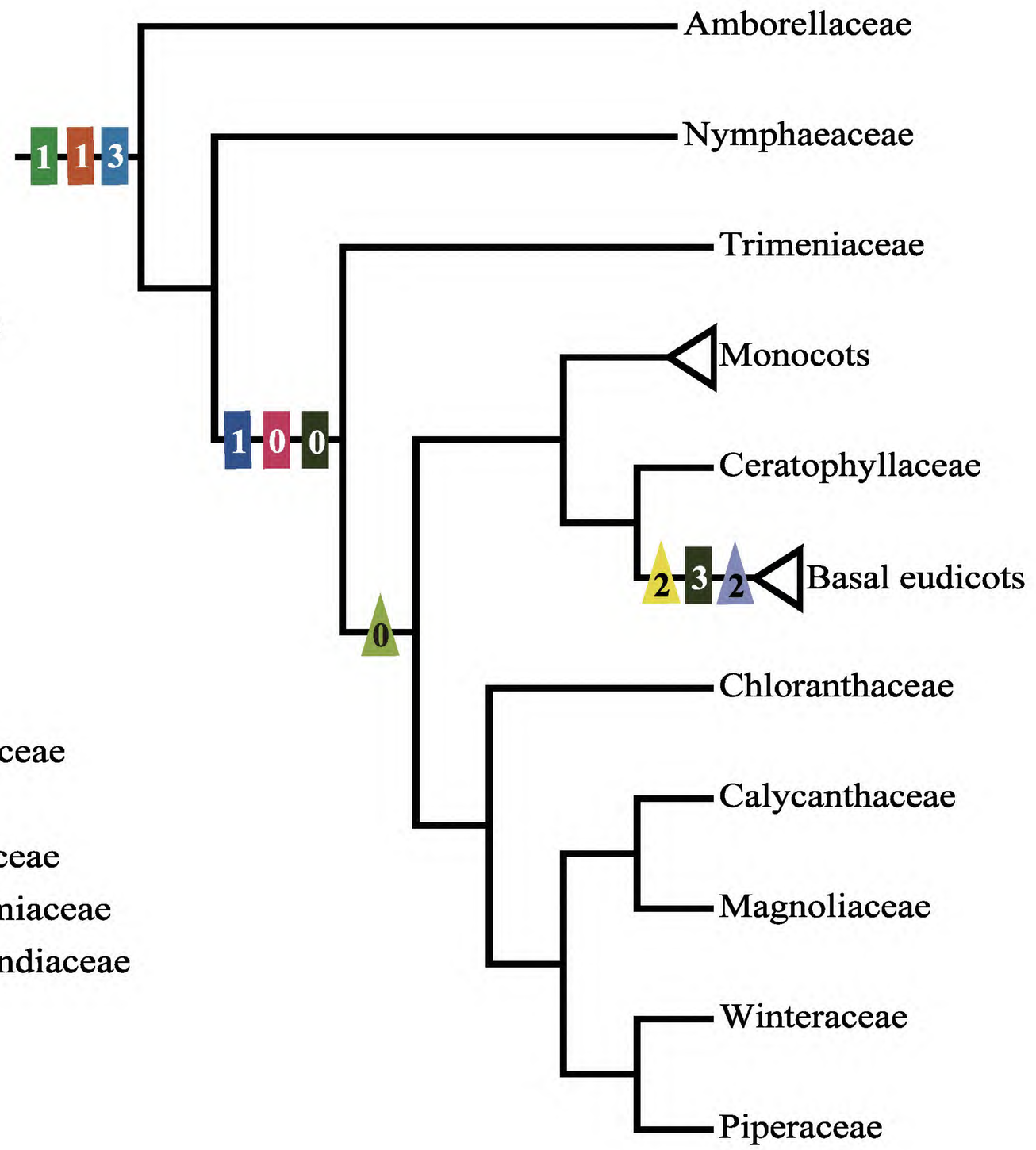


Figure 9. Synapomorphies and likely synapomorphies inferred from a comprehensive dataset of pollen characters analyzed with Fitch parsimony, labeled on the branches of a range of phylogenetic topologies for basal angiosperms. Topologies in this figure are from Qiu et al. (2006, eight genes), a modified combination of Jansen et al. (2007) and Moore et al. (2007), Soltis et al. (2007), and Soltis et al. (2011). Synapomorphies are shown as rectangles and likely synapomorphies as triangles, with shape color indicating pollen characters and the number within indicating character state change, following Appendix 2. As shown in Appendix 2, pollen character 10, aperture membrane, is not shown because no synapomorphies were found for this character.

12. Soltis et al. (2011)

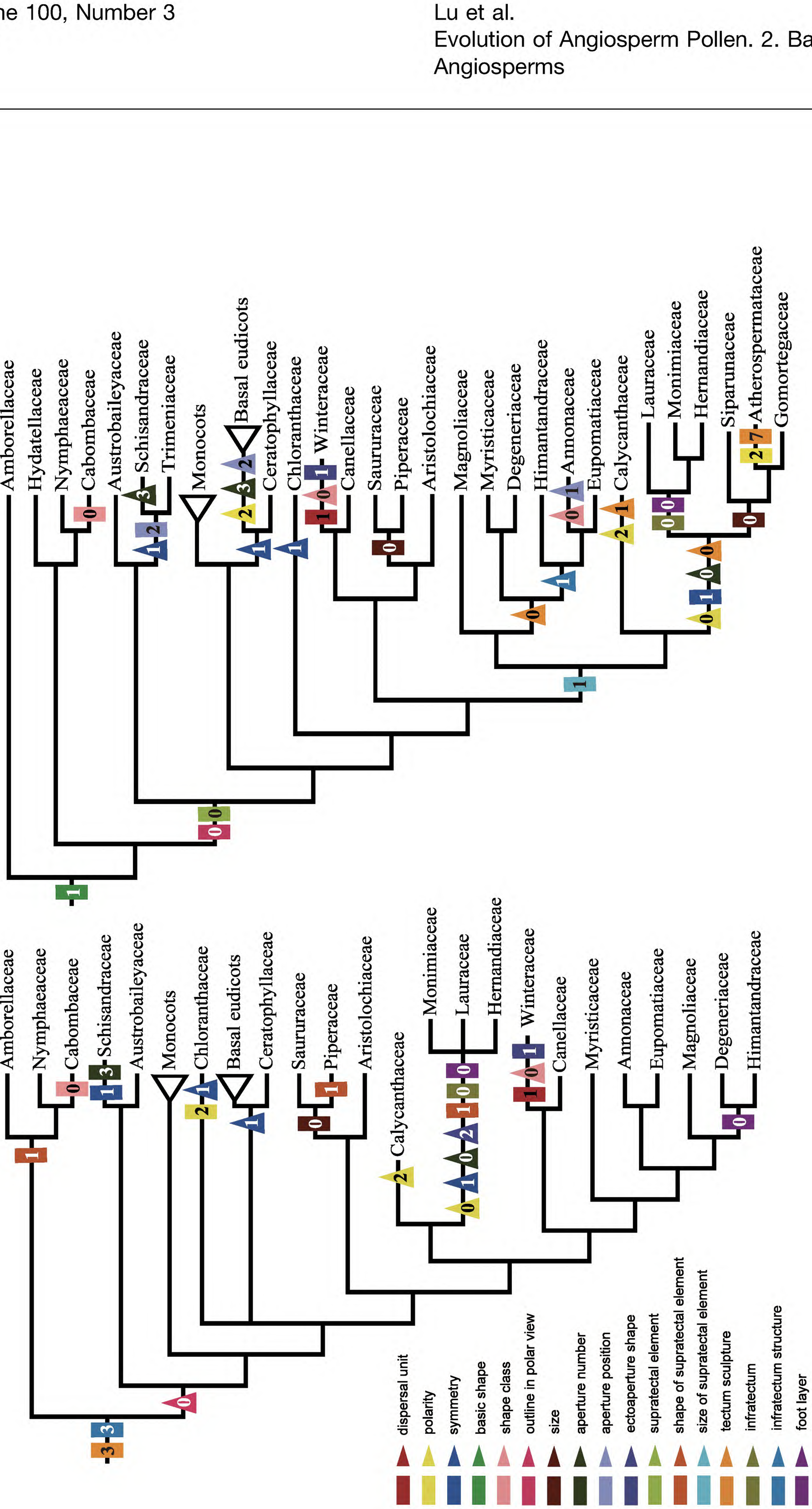


Figure 9. Continued.

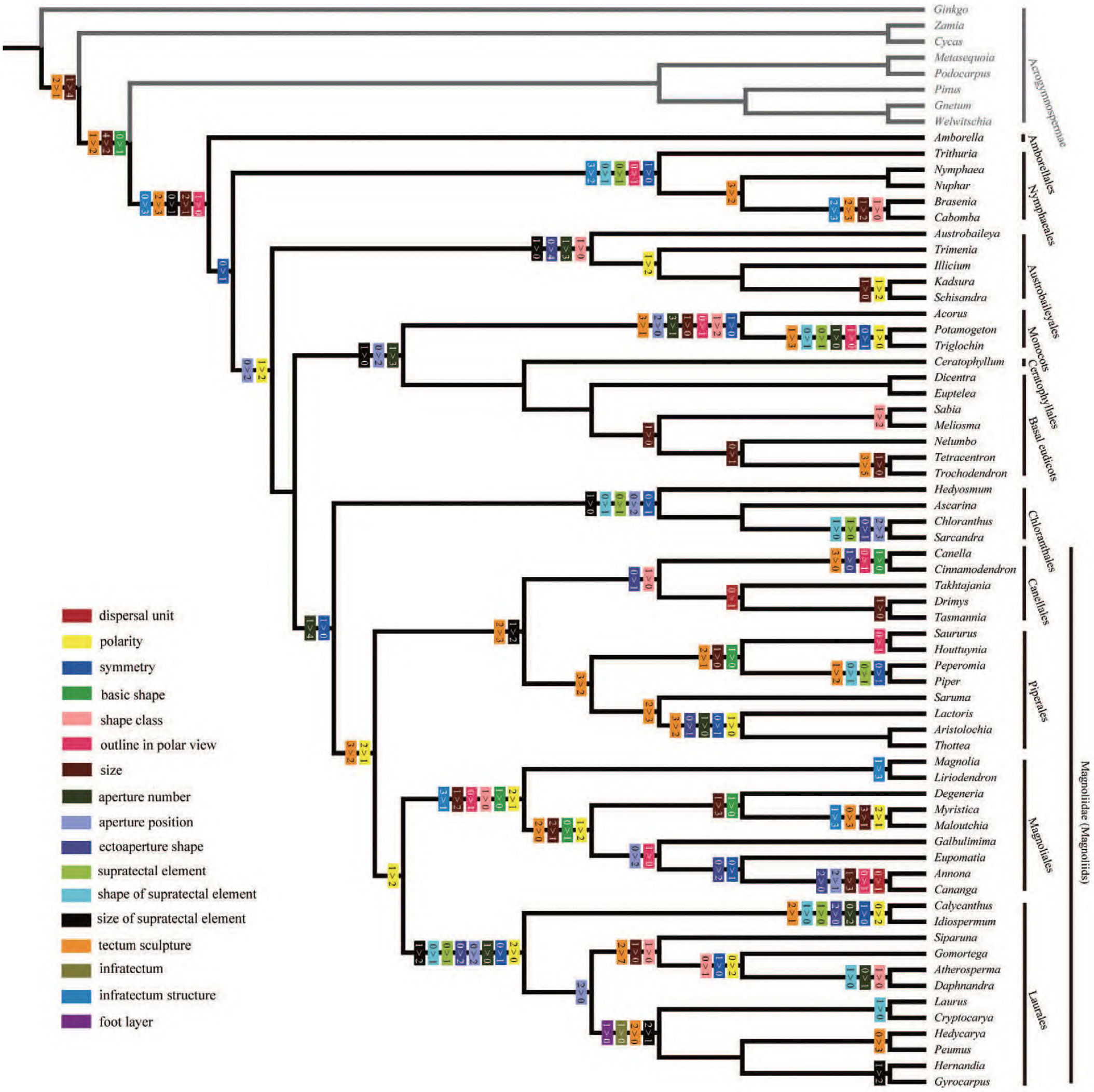


Figure 10. Inferences of unambiguous character state changes for the 18 pollen characters analyzed in this study, reconstructed using hierarchical Bayesian inference, with democratic coding method on a maximum likelihood (DML) phylogeny generated based on the molecular data of Soltis et al. (2011). Changes are mapped assuming a single transition between differing states at adjacent nodes. Pollen characters and the numbers within indicate character state changes, following Appendix 2. As shown in Appendix 2, pollen character 10, aperature membrane, is not shown because no changes were found for this character.

The new tree was reconstructed using maximum likelihood method (ML) with RAxML (Stamatakis, 2006). A bootstrap analysis (1000 replicates) was performed simultaneously with the ML analysis (option, -f a) under the GTRGAMMA model. Because the topology of the generated tree differed slightly from that of Soltis et al. (2011), we used constraints to ensure a match to Soltis et al.

POLLEN CHARACTERS AND CODING STRATEGY

Pollen morphological data were taken from the literature (mostly SEM and transmission electron microscopy [TEM] data), from websites, such as PalDat – Palynological Database, an Online Publication on fossil and recent Pollen and Spores (<http://www.paldat.org/>), and from the new observations presented here. Literature-based data were extracted directly from published images and measurements rather than descriptions to avoid discrepancies due to misinterpretation and conflicting terminology. The terminology used here follows that of Wortley et al. (2015) and Punt et al. (2007). Eighteen pollen characters were scored (cf. Appendix 2 for the coded character states): dispersal unit,

polarity, symmetry (in polar view), basic shape (in equatorial view), shape class, outline in polar view (amb), size (diameter of largest axis), aperture number, aperture position, aperture membrane, ectoaperture shape, supratectal element presence, shape of supratectal elements, size of supratectal elements, tectum sculpture, infratectum presence, infratectum structure, and foot layer presence. These cover all the variable and heritable characters commonly noted in LM, SEM, and TEM observations in previous and present studies of basal angiosperms. Coding strategy followed that of Wortley et al. (2015), with the exception of supratectal elements, for which a size division was defined at 0.5 μm in this study, because this minimized instances of polymorphic data points for our taxa more effectively than a 1-μm boundary. Characters were treated as unordered, binary or multistate characters, with inapplicable states coded as "-" and unknown data as "?". We generated both comprehensive (including polymorphic states; 78 genera of basal angiosperms, gymnosperms, monocots, and basal eudicots from all 12 studied topologies) and democratic (removing polymorphic states by coding for the most common state; including 68 genera of basal angiosperms, gymnosperms, monocots, and basal eudicots from the topology of Soltis et al. [2011]) matrices according to Wortley et al. (2015). In coding democratic states for size of supratectal element, those with both size $<$ 0.5 μm and $\geq$ 0.5 μm were evenly distributed on a grain as a state. Data matrices are shown in Tables 1 and 2.

METHODS FOR ANALYZING CHARACTER EVOLUTION

We used two strategies for optimization of pollen characters to infer ancestral states and synapomorphic traits, and possible evolutionary history: (1) analysis of the comprehensive dataset with Fitch parsimony on multiple phylogenetic estimates (hereafter abbreviated as CFP), and (2) analysis of the democratic dataset with Fitch parsimony, maximum likelihood, and hierarchical Bayesian inference (hereafter abbreviated as DFP, DML, and DHB, respectively).

Because phylogenetic estimates for the basal angiosperms remain controversial, the first strategy optimized the comprehensive dataset using Fitch parsimony (CFP) onto 12 trees with alternative positions of the major early-diverging lineages, using Mesquite 2.75 (Maddison & Maddison, 2011).

Because the above topologies were branch-length free, they could only be analyzed by non-model-based methods (i.e., parsimony). In order to perform model-based methods (ML and Bayesian methods), we also optimized pollen data for the 18 characters upon the new ML tree of the 64 taxa based on the data of Soltis et al. (2011). Because ML methods are presently not applicable to datasets including polymorphic data points, these analyses were conduced on a democratic matrix. Characters were then optimized, using Fitch parsimony (FP), maximum likelihood (ML), and Bayesian inference (HB) on this ML tree. FP and ML optimizations (using the Mk-1 model) were conducted in Mesquite 2.75; HB optimization was performed by using BayesMultistate in Bayestraits 1.0 (available from <http://www.evolution.rdg.ac.uk>) and implementing the reversible-jump hyperprior method. An exponential prior distribution was found to be most appropriate for all characters (seeded from a uniform 0–10 distribution, 0–30 distribution, or 0–40 distribution depending on the character). We used rate variations ranging from nine to 300 (set to obtain the acceptance value between 20% and 40%), a sampling frequency of 300 generations, and a burn-in period of 10,000 generations for a total of 5,000,000 generations. For ML and HB analyses, we took the state with the highest likelihood or probability at each node to represent the state of this node in phylogenetic reconstructions. If the likelihood or probability of the most likely state was found to be $\geq$ 95%, this state was considered to be strongly indicated as the likely reconstructed state for the node.

In this study we highlight both synapomorphies and likely synapomorphies. A likely synapomorphy is defined as a derived character state shared by all included taxa and their most recent common ancestor, in instances where the next node subtending the node of their most recent common ancestor underwent an ambiguous change. In such instances, this character state could be reconstructed as both a synapomorphy for the clade comprising these taxa, or for a larger clade that comprised these taxa and all their sister groups under the Most Parsimonious Reconstruction (MPR) mode implemented in Mesquite 2.75 (Lu et al., 2009, 2010).

Results

PALYNOLOGICAL OBSERVATIONS

Pollen morphology of 34 species of 31 genera in 23 families observed under LM and SEM were described below and presented to show all 18 diverse pollen characters and their states in the basal angiosperms. Pollen SEM images from previous literature of three species, i.e., *Degeneria vitiensis* I. W. Bailey & A. C. Sm., *Gomortega keule* (Molina) Baill., and *Lactoris fernandeziana* Phil. (see below), for which pollen

Table 1. Comprehensive data matrix of pollen morphological characters for basal angiosperms (see text for details).

Acorus L.	01012101010000-1131
Akebia Decne.	0211200321000-{1,3}131
Amborella Baill.	0{0,1}{0,1}1{0,1}1{0,1}{0,1}01{0,1}1106131
Anemopsis Hook. & Arn.	0100-00101000-{1,2}131
Annona L.	{0,1}10{0,1}01{1,2,3}{0,1}1-{0,1}00-{1,2,3}131
Aristolochia L.	0{0,1}11{0,1}{0,1}{1,2}{0,1}0?{0,1}00-{0,1,2,3}131
Asarum L.	0211{0,1}0{1,2}{0,2,3,4}21{0,1,2}101{1,2}13{0,1}
Ascarina J. R. Forst. & G. Forst.	0111{0,1}0{0,1}10101103131
Atherosperma Labill.	0201{0,1}{0,1}{0,1}{1,2}{0,1}1{0,2}10{0,1}7131
Austrobaileya C. T. White	0101101101000-{2,3}131
Brasenia Schreb.	0101011101011031{2,3}1
Cabomba Aubl.	010101{1,2}101010-1131
Calycanthus L.	020{0,1}10{1,2}{1,2}{0,2}1000-{1,2,3}131
Cananga (DC.) Hook. f. & Thomson	1211013{0,1}{1,2}1{0,1,2}00–0111
Canella P. Browne	{0,1}100-{0,1}1101000-{0,1,3}131
Ceratophyllum L.	0011{1,2}0{0,1}0—00-00-0
Chimonanthus Lindl.	0201{0,1}11221000-{1,2,3}131
Chloranthus Sw.	021110{0,1}421{0,1}1103131
Cinnamodendron Endl.	0100-{0,1}1101000-0131
Cinnamomum Schaeff.	001110{0,1,2}0—11{0,1}10-0
Cissampelos L.	0211{0,1}00320000-3131
Cryptocarya R. Br.	0011{0,1}{0,1}10—{0,1}01{0,2}0-0
Cycas L.	0100-{0,1}{1,2}101000-{0,1,3}101
Daphnandra Benth.	0201{0,1}{0,1}{0,1}1{0,1}1{0,2}10{0,1}7131
Degeneria I. W. Bailey & A. C. Sm.	020{0,1}112101000-01{1,2}0
Dicentra Bernh.	0211{1,2}0{0,1}{3,4}21{0,4}00-{1,3}131
Doryphora Endl.	0201{0,1}{0,1}{0,1}{1,2}{0,1}1{0,2}10{0,1}7131
Drimys J. R. Forst. & G. Forst.	110100{0,1}101100-3131
Eupomatia R. Br.	0211{0,1}01121200-01{1,2}0
Euptelea Siebold & Zucc.	0211{0,1,2}01{3,4}{2,3}1000–3131
Galbulimima F. M. Bailey	0001101101000-{0,2}1{1,2}0
Ginkgo L.	010{0,1}1{0,1}1101000-2101
Gnetum L.	00111{0,1}00—11{0,1}2110
Gomortega Ruiz & Pav.	00111000—1102131
Gyrocarpus Jacq.	00111010—{0,1}1{0,1}00-0
Hedycarya J. R. Forst & G. Forst.	{0,1}{0,1}{0,1}1{0,1}{0,1}{0,1}{0,1}012110{1,2}0-0
Hedyosmum Sw.	0211101{1,4}{0,2}101103131
Hernandia L.	00111020—11{0,1}00-0
Hortonia Wight & Arn.	021110{0,1}0—11050-0
Houttuynia Thunb.	0100-00101000-{1,2}131
Idiospermum S. T. Blake	0201{0,1}1122-000-1??1
Illicium L.	0211{0,1}01321{0,4}00–3131
Kadsura Juss.	0111{0,1}0{0,1}3{0,2}1{0,4}00–3131
Knema Lour.	010{0,1}11{1,2}101{0,1}00-{2,3}131
Lactoris Phil.	1101{0,1}{0,1}0101{0,1}00–21{1,3}1
Laurus L.	001110{0,1}0—11{0,1}00-0
Liriodendron L.	0100-1{1,2}101000-{1,2,3}131
Magnolia L.	0100-1{1,2}1010{0,1}01{1,2}1{1,2,3}1
Mahonia Nutt.	00–110{1,2}{3,4}31{0,3,4}00-{1,2,3}131
Maloutchia Warb.	010{0,1}11{0,1,2}101{0,1}111{1,2}1{1,2,3}1
Meliosma Blume	0211{1,2}00321000-{1,3}131
Metasequoia Hu & W. C. Cheng	010110{0,1}0—00–110
Myristica Gronov.	010{0,1}11{1,2}101000-{2,3}131
Nelumbo Adans.	0{1,2}1110{1,2}{1,2,3,4}{0,2}1{0,2}00-{2,3}131
Nuphar Sm.	010{0,1}11110101112l2{0,1}
Nymphaea L.	0{1,2}{0,1}{0,1}1{0,1}{1,2}1{0,2}{0,1}{0,2}11{0,1}{0,3}1{1,2,3}{0,1}
Peperomia Ruiz & Pav.	0011100{0,1}01{0,1}1104131
Peumus Molina	00111010—11130-0
Pinus L.	0100–121000100{2,3}101

Table 1. Continued.

Piper L.	01{0,1}1{0,1}{0,1}0{0,1}01011{0,1}{0,1,2}131
Podocarpus L'Hér. ex Pers.	0100-{0,1}{1,2}10101102101
Potamogeton L.	0011{0,1}0{0,1}0—1103131
Sabia Colebr.	0211{1,2}00321000-{1,3}131
Sarcandra Gardner	0211{1,2}01{0,4}3?100-3131
Saruma Oliv.	0101{0,1}11{0,1}01000-{0,1,2,3}131
Saururus L.	0100-{0,1}0101000-{1,2}131
Schisandra Michx.	0111{0,1}0{0,1}3{0,2}1{0,4}00–3131
Siparuna Aubl.	0011{0,1}{0,1}00—11101{1,2}1
Takhtajania Baranova & J.-F. Leroy	1101001101100-{1,2,3}131
Tasmannia R. Br. ex DC.	110100{0,1}101100-3131
Tetracentron Oliv.	0211100321000-5131
Thottea Rottb.	0011{0,1}{0,1}{1,2}0—00–113{0,1}
Triglochin L.	001110{0,1}0—110313{0,1}
Trimenia Seem.	0211{1,2}00{0,2,4}{2,3}1{0,1}{0,1}10{1,2,3}131
Trithuria Hook. f.	010{0,1}1{0,1}{0,1}10101101131
Trochodendron Siebold & Zucc.	0211{0,1}00321000-3131
Welwitschia Hook. f	0100-1{1,2}100000-5110
Zamia L.	010{0,1}114101000-1101

samples were unavailable, were permitted to be reprinted in our study (cf. Figs. 1–6).

Amborella trichopoda Baill. (Amborellales, Amborellaceae, Fig. 1A, B) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (oblate to subspheroidal); amb usually elliptic; size small to medium (ca. 15–30 μm); monoporate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements spinulose; tectum unique, *Amborella*-type.

Trithuria australis (Diels) D. D. Sokoloff., Remizowa, T. D. Macfarl. & Rudall (Nymphaeales, Hydatellaceae, Fig. 1C) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped or globose (spheroidal); amb elliptic to circular; size small to medium (ca. 12–27 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements spinulate; tectum perforate.

Nymphaea alba L. (Nymphaeales, Nymphaeaceae, Fig. 1D, E) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (oblate to spheroidal); amb elliptic to circular; size small to medium (ca. 23–32 μm); zonate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements spinulate and verrucate; tectum imperforate.

Nuphar luteum Walp. (Nymphaeales, Nymphaeaceae, Fig. 1F) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (oblate to subspheroidal); amb usually elliptic; size medium (ca. 35–42 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements spinulate and verrucate; tectum rugulate.

Austrobaileya scandens C. T. White (Austrobaileyales, Austrobaileyaceae, Fig. 1G–I) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (spheroidal); amb usually circular; size medium (ca. 38–45 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum reticulate.

Trimenia moorei (Oliv.) Philipson (Austrobaileyales, Trimeniaceae, Fig. 1J–L) has pollen dispersed as monads, isopolar or subisopolar, radially symmetrical; globose (prolate); amb circular; size small (ca. 10–15 μm); inaperturate or bi-colpate, apertures located at the equator, with sculptured aperture membrane; supratectal elements spinulose; tectum reticulate.

Illicium micranthum Dunn (Austrobaileyales, Schisandraceae, Fig. 2A, B) has pollen dispersed as monads, isopolar, radially symmetrical; globose (oblate); amb circular; size medium (ca. 45–50 μm); trisyncolpate, apertures centered at the equator, with sculptured aperture membrane; tectum reticulate.

Illicium macranthum A. C. Sm. (Austrobaileyales, Schisandraceae, Fig. 2C) has pollen dispersed as monads, isopolar, radially symmetrical; globose (oblate); amb circular; size medium (ca. 25–30 μm); trisyncolpate, apertures located at the equator, with sculptured aperture membrane; tectum reticulate.

Chloranthus japonicus Siebold (Chloranthales, Chloranthaceae, Fig. 2D) has pollen dispersed as monads, isopolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 15–20 μm); hexa-colpate, apertures located at the equator,

Table 2. Democratic data matrix of pollen morphological characters for basal angiosperms (see text and Table 2 for details).

Acorus L.	01012101010000-1131
Amborella Baill.	0101010101111106131
Annona L.	110101211-000-2131
Aristolochia L.	001110100?-00-2131
Ascarina J. R. Forst. & G. Forst.	011110010101103131
Atherosperma Labill.	02010001–121027131
Austrobaileya C. T. White	01011011010000-2131
Brasenia Schreb.	0101011101011031 31
Cabomba Aubl.	01010121010101-1131
Calycanthus L.	02011012210001-1131
Cananga (DC.) Hook. f. & Thomson	121101311-000-0111
Canella P. Browne	0100-11101000-3131
Ceratophyllum L.	00111010—00-00-0
Chloranthus Sw.	021110142101103131
Cinnamodendron Endl.	0100-11101000-0131
Cryptocarya R. Br.	00111010—10120-0
Cycas L.	0100-11101000-1101
Daphnandra Benth.	02010001–121027131
Degeneria I. W. Bailey & A. C. Sm.	0200-12101000-0110
Dicentra Bernh.	0211100321000-3131
Drimys J. R. Forst. & G. Forst.	1101000101100-3131
Eupomatia R. Br.	0211001121200-0110
Euptelea Siebold & Zucc.	0211101321000-3131
Galbulimima F. M. Bailey	00011011010000-2110
Ginkgo L.	0100-11101000-2101
Gnetum L.	00111100—1122110
Gomortega Ruiz & Paz.	00111000—1102131
Gyrocarpus Jacq.	00111010—11200-0
Hedycarya J. R. Forst & G. Forst.	00111000012110 10-0
Hedyosmum Sw.	021110142101103131
Hernandia L.	00111020—11200-0
Houttuynia Thunb.	0100-00101000-1131
Idiospermum S. T. Blake	020111122-000-1??1
Illicium L.	0211001321400-3131
Kadsura Juss.	0111000321400-3131
Lactoris Phil.	1101100101100-2111
Laurus L.	00111010—11200-0
Liriodendron L.	0100-12101000-2131
Magnolia L.	0100–1210101011131
Maloutchia Warb.	0100–1110111112121
Meliosma Blume	0211200321000-3131
Metasequoia Hu & W. C. Cheng	01011010—00–110
Myristica Gronov.	0100-11101000-3131
Nelumbo Adans.	0211101321000-3131
Nuphar Sm.	0101111101011121 21
Nymphaea L.	0201101100211231 21
Peperomia Ruiz & Pav.	0011100001-1104131
Peumus Molina	00111010—11130-0
Pinus L.	0100–1210001002101
Piper L.	011110010101122131
Podocarpus L'Hér. ex Pers.	0100–1210101102101
Potamogeton L.	00111000—1103131
Sabia Colebr.	0211200321000-3131
Sarcandra Gardner	021110143?100-3131
Saruma Oliv.	0101111101000-3131
Saururus L.	0100-10101000-1131
Schisandra Michx.	0111000321400-3131
Siparuna Aubl.	00110000—1110121
Takhtajania Baranova & J.-F. Leroy	1101001101100-3131
Tasmannia R. Br. ex DC.	1101000101100-3131
Tetracentron Oliv.	0211100321000-5131
Thottea Rottb.	00111010—00–1131
Triglochin L.	00111000—1103131
Trimenia Seem.	0211200221111102131
Trithuria Hook. f.	0101100101011011 31
Trochodendron Siebold & Zucc.	0211100321000-3131
Welwitschia Hook. f	0100-12100000-5110
Zamia L.	0100114101000-1101

with sculptured aperture membrane; tectum reticulate.

Chloranthus holostegius (Hand.-Mazz.) S. J. Pei & Shan (Chloranthales, Chloranthaceae, Fig. 2E, F) has pollen dispersed as monads, isopolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 13–16 μm); hexa-colpate, apertures located at the equator, with sculptured aperture membrane; tectum reticulate.

Canella winterana (L.) Gaertn. (Canellales, Canellaceae, Fig. 2G–I) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped or globose (oblate); amb elliptic; size medium (ca. 28–33 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate.

Drimys piperita Hook. f. (Canellales, Winteraceae, Fig. 2J–L) has pollen dispersed as tetrads, radially symmetrical, globose (spheroidal), size of each grain small to medium (ca. 22–30 μm); the individual grains each heteropolar, bilaterally symmetrical; globose (oblate); amb circular; monoporate, the aperture located at the distal pole, with sculptured aperture membrane; tectum reticulate.

Hydnora abyssinica A. Braun (Piperales, Hydnoraceae, Fig. 3A) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (oblate to subspheroidal); amb circular; size small (ca. 20–25 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements verrucate; tectum rugulate.

Lactoris fernandeziana (Piperales, Aristolochiaceae, Fig. 3B–D), reprinted SEM images from Zavada and Taylor (1986), has pollen dispersed as tetrads, radially symmetrical and globose (spheroidal), the size of each grain small (ca. 15 μm); the individual grains heteropolar and bilaterally symmetrical; globose (oblate); amb elliptic or circular; monoporate or monocolpate, aperture located at the distal pole, with

sculptured aperture membrane; tectum microrugulate.

Aristolochia contorta Bunge (Piperales, Aristolochiaceae, Fig. 3E, F) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (spheroidal); amb circular; size medium (ca. 27–30 μm); monoporate, aperture located at the distal pole, with sculptured aperture membrane; tectum rugulate.

Peperomia heyneana Miq. (Piperales, Piperaceae, Fig. 3G) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 5–8 μm); inaperturate; supratectal elements spinulose; tectum areolate.

Saururus chinensis (Lour.) Baill. (Piperales, Saururaceae, Fig. 3H) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped; amb elliptic; size small (ca. 10–15 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate.

Houttuynia cordata Thunb. (Piperales, Saururaceae, Fig. 3I) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped; amb elliptic; size small (ca. 18–20 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate.

Liriodendron tulipifera L. (Magnoliales, Magnoliaceae, Fig. 3J) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped; amb elliptic; size large (ca. 50–57 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate to finely reticulate.

Magnolia grandiflora L. (Magnoliales, Magnoliaceae, Fig. 3K, L) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped; amb elliptic; size large (ca. 78–85 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate.

Degeneria vitiensis (Magnoliales, Degeneriaceae, Fig. 4A–C), reprinted SEM images from Sampson (2000a), has pollen dispersed as monads, isopolar, bilaterally symmetrical; boat-shaped or globose (subspheroidal); amb elliptic; size large (ca. 50–60μm); monocolpate, aperture located at the distal pole, sometimes nearly encircling the grain, with sculptured aperture membrane; tectum imperforate.

Knema globularia (Lam.) Warb. (Magnoliales, Myristicaceae, Fig. 4D, E) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (subspheroidal); amb elliptic; size medium (ca. 25–28 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum reticulate.

Myristica Gronov., sp. indet. (Magnoliales, Myristicaceae, Fig. 4F) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (subspheroidal); amb elliptic; size medium (ca. 25–30 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum rugulate.

Galbulimima baccata F. M. Bailey (Magnoliales, Himantandraceae, Fig. 4G, H) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (subspheroidal); amb circular; size medium (ca. 38–43 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum rugulate.

Eupomatia laurina R. Br. (Magnoliales, Eupomatiaceae, Fig. 4I–K) has pollen dispersed as monads, isopolar, radially symmetrical; globose (spheroidal); amb circular; size medium (ca. 43–47 μm); zonate, the aperture located at the equator, with sculptured aperture membrane; tectum imperforate (psilate).

Annona muricata L. (Magnoliales, Annonaceae, Fig. 4L) has pollen dispersed as tetrads, radially symmetrical and globose (spheroidal), size of each grain very large to gigantic (ca. 160–220 μm); the individual grains heteropolar and bilaterally symmetrical; globose (oblate); amb elliptic; monoporate, the aperture located at the proximal pole; tectum reticulate.

Annona glabra L. (Magnoliales, Annonaceae, Fig. 5A, B) has pollen dispersed as tetrads, radially symmetrical and globose (spheroidal), size of each grain medium to large (ca. 45–65 μm); the individual grains heteropolar and bilaterally symmetrical; globose (oblate); amb elliptic; monoporate, the aperture located at the proximal pole; tectum perforate.

Cananga odorata (Lam.) Hook. f. & Thomson var. *fruticosa* (Craib) J. Sinclair (Magnoliales, Annonaceae, Fig. 5C) has pollen dispersed as monads, isopolar, radially symmetrical; globose (oblate); amb elliptic; size very large (ca. 105–110 μm); zonate, the aperture located at the equator, with sculptured aperture membrane; tectum perforate.

Calycanthus chinensis (W. C. Cheng & S. Y. Chang) P. T. Li (Laurales, Calycanthaceae, Fig. 5D–F) has pollen dispersed as monads, isopolar, bilaterally symmetrical; globose (oblate); amb elliptic; size medium to large (ca. 45–52 μm); bi-colpate, apertures located at the equator, with sculptured aperture membrane; tectum rugulate.

Siparuna guianensis Aubl. (Laurales, Siparunaceae, Fig. 5G, H) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 14–16 μm); inaperturate; supratectal elements spinulate; tectum imperforate.

Gomortega keule (Laurales, Gomortegaceae, Fig. 5I–K), reprinted SEM images from Hesse and Kubitzki (1983), has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 23 μm); inaperture; supratectal elements spinulate; tectum rugulate.

Atherosperma moschatum Labill. (Laurales, Atherospermataceae, Figs. 5L, 6A) has pollen dispersed as monads, isopolar, bilaterally symmetrical; globose (oblate); amb elliptic; size medium (ca. 33–37 μm); bi-colpate, apertures located at distal and proximal poles, with sculptured aperture membrane; supratectal elements verrucate; tectum rudimentary.

Doryphora sassafras Endl. (Laurales, Atherospermataceae, Fig. 6B) has pollen dispersed as monads, isopolar, bilaterally symmetrical; globose (oblate); amb elliptic; size medium (ca. 40–44 μm); bi-colpate, apertures located at distal and proximal poles, with sculptured aperture membrane; supratectal elements verrucate; tectum rudimentary.

Daphnandra tenuipes G. Perkins (Laurales, Atherospermataceae, Fig. 6C) has pollen dispersed as monads, isopolar, bilaterally symmterical; globose (oblate); amb elliptic; size medium (ca. 37–40 μm); bi-colpate, apertures located at distal and proximal poles, with sculptured aperture membrane; supratectal elements verrucate; tectum rudimentary.

Laurus nobilis L. (Laurales, Lauraceae, Fig. 6D, E) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small to medium (ca. 22–25 μm); inaperturate; supratectal elements spinulate and verrucate; tectum imperforate.

Hernandia didymantha Donn. Sm. (Laurales, Hernandiaceae, Fig. 6F, G) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size large (ca. 50–55 μm); inaperturate; supratectal elements spinulate and verrucate; tectum imperforate.

Peumus boldus Molina (Laurales, Monimiaceae, Fig. 6H, I) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size medium (ca. 26–30 μm); inaperturate; supratectal elements spinulate and verrucate; tectum reticulate.

Ceratophyllum,sp. indet. (Ceratophyllales, Ceratophyllaceae, Fig. 6J–L) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 7–10 μm); inaperturate; exine nearly psilate.

POLLEN DIVERSITY

The pollen grains of basal angiosperms display a number of widely shared characteristics, including dispersal as monads, sculptured apertural membranes (if apertures are present), and an exine that comprises a tectum and infratectum plus a foot layer (except for Laurales). The taxa studied also display high diversity in pollen size, aperture number, aperture position, ectoaperture shape, tectum sculpture, and infratectal structure. Genera such as *Annona* L., *Aristolochia* L., *Asarum* L., *Calycanthus* L., *Hedycarya* J. R. Forst. & G. Forst., *Maloutchia*, *Nymphaea* L., and *Saruma* Oliv. showed greater infrageneric pollen variation than other studied genera, whereas *Drimys* J. R. Forst. & G. Forst., *Houttuynia* Thunb., *Laurus* L., and *Magnolia* L. were more consistent. The level of variation did not seem to depend, at generic level, upon taxonomic diversity, e.g., pollen grains of the medium-sized genus *Nymphaea* (ca. 50 species) were highly variable, whereas those of the large genus *Magnolia* (ca. 210 species) were relatively homogeneous. At family level, certain families, including the Aristolochiaceae, Myristicaceae, and Nymphaeaceae, showed more palynological variation than others such as the Hernandiaceae, Lauraceae, Saururaceae, Schisandraceae, and Winteraceae.

Supratectal elements, in particular, were highly diverse in the basal angiopserms. Based on our observations, five main types of character combinations were present: (1) sparsely large spinulose (with large, pointed, well-separated spines, commonly surrounded by tiny granules; cf. Fig. 6E, G, I); (2) densely large spinulose (large, pointed, closely packed spines, usually with expanded bases; cf. Fig. 5H); (3) small spinulose (small spinules present on a rugulate, reticulate, striate or *Amborella*-type tectum; cf. Fig. 1B, L); (4) verrucate (Fig. 6C); and (5) striate. Most taxa in the Laurales and Nymphaeaceae displayed the sparsely large spinulose type, and in the Piperaceae, the densely large spinulose type. The Amborellales, Chloranthales, and Hydatellaceae were characterized by the small spinulose type, the Atherospermataceae by the verrucate type, and *Cabomba* Aubl. by the striate type.

ANCESTRAL STATE RECONSTRUCTION

Using comprehensive coding with Fitch parsimony (CFP), a total of 216 character reconstructions (18 characters across 12 tree topologies) were conducted (Figs. 7–9). These results imply a number of plesiomorphic states for angiosperms, with several of them occurring on all 12 tree topologies, such as dispersal as monads, the pollen grains heteropolar, spheroidal in shape, monoaperturate, aperture distal, colpate, aperture membrane sculptured, supratectal element size < 0.5 μm, infratectum present, and foot

layer present. A further three character states were reconstructed as unambiguously plesiomorphic for angiosperms in most (10 or 11) topologies: pollen symmetry bilateral, outline in polar view elliptic, and pollen size medium (25–49 μm). Certain characters did not produce an unambiguously reconstructed state at the root of the angiosperms on most topologies: basic pollen shape, supratectal element presence and supratectal element shape, as well as tectum sculpture and infratectum structure.

Seventeen out of the 18 characters studied, the exception being aperture membrane ornamentation, were inferred to provide a synapomorphy or likely synapomorphy for at least one lineage of the basal angiosperms, across the various possible tree topologies, using CFP (Figs. 7–9). Among those characters, pollen polarity, symmetry, aperture number, supratectal element presence, supratectal element size, and tectum sculpture provided synapomorphies or likely synapomorphies for more than five families or higher lineages of basal angiosperms, whereas pollen dispersal unit, basic shape, outline in polar view, ectoaperture shape, supratectal element shape, infratectum, and infratectum structure provided synapomorphies or likely synapomorphies for only one or two evaluated families or higher lineages. Based on these analyses, 36 families or higher lineages could be defined by synapomorphies or likely synapomorphies in pollen characters. The following groups were defined by at least three pollen synapomorphies or likely synapomorphies in our sample: (1) angiosperms; (2) Atherospermataceae; (3) the clade comprising the Austrobaileyales and all later-diverging angiosperms; (4) basal eudicots; (5) Calycanthaceae; (6) Chloranthales; (7) the clade comprising Hernandiaceae, Lauraceae, and Monimiaceae; (8) the clade of Laurales with Calycanthaceae excluded; (9) the clade comprising Laurales and Magnoliales; and (10) Winteraceae. The clade comprising the Hernandiaceae, Lauraceae, and Monimiaceae was defined by at least five pollen synapmorphies or likely synapomorphies, and the clade of Laurales with Calycanthaceae excluded was defined by nine. Other lineages, such as the Austrobaileyales, Cabombaceae, Laurales, Nymphaeaceae, and Saururaceae, displayed only one palynological synapomorphy or likely synapomorphy. Across the 12 studied topologies (cf. Figs. 7–9 herein), that of Qiu et al. (2006, three genes) was reconstructed with 22 synapomorphies plus 14 likely synapomorphies (36 in total) for related taxa. The topologies from Qiu et al. (1999, 2000, 2005, 2006, eight genes) and Soltis et al. (2000, 2007, 2011) were reconstructed with a total of 20 to 32 synapomorphies plus likely synapomorphies. The trees from Graham and Olmstead (2000); Mathews and Donoghue (2000); Müller et al. (2006); and Jansen et al. (2007) and Moore et al. (2007) displayed only a few synapomorphies or likely synapomorphies (two, four, nine, and 10, respectively) due to the lower number of taxa, and therefore nodes, in these trees.

Fourteen of the 18 pollen characters also displayed unambiguous plesiomorphic states for the root of the angiosperms with democratic coding under Fitch parsimony (DFP), maximum likelihood (DML), and hierarchical Bayesian optimization (DHB). These reconstructed plesiomorphic states were simlar to the CFP analysis, with the exception of supratectal element size (< 0.5 μm) and pollen size medium (25–49 μm), as well as supratectal elements verrucate, the tectum sculputure rugulate, and the infratectum structure alveolate. Unambiguous or probable plesiomorphic states for the other four characters were not consistently reconstructed across the three methods. For instance, basic shape was reconstructed as boat-shaped using DFP and DML, but as globose using DHB. Ancestral pollen size was recovered as medium using DML, but large using DHB, and ambiguous under DFP. Supratectal elements were reconstructed as present using DFP, absent under DML, and ambiguous under DHB, and the size of supratectal elements was < 0.5 μm, using DFP and DHB, but ambiguous under DML.

We compared the results of democratic Fitch parsimony (DFP), democratic maximum likelihood (DML), and democratic Bayesian inference (DHB) to examine differences in character evolutionary patterns inferred by the three methods (using the most probable state found at each node in DML and DHB). Among the 18 characters studied, only dispersal unit evolved unequivocally at all nodes with all three methods. Pollen polarity, symmetry, basic shape, outline in polar view, aperture number, shape of supratectal element, and foot layer displayed a number of nodes with equivocal changes in DFP; pollen infratectum only in DML; pollen size in both DFP and DHB; aperture membrane, supratectal element presence, and size of supratectal element in both DML and DHB; and the rest of the 18 characters, in all three methods. Generally, DFP generated more ambiguous states for a character than DML or DHB, but for those characters with a number of inapplicable states (gaps), such as supratectal element size, DML and DHB tended to generate more states with equal possibility at a node. Synapomorphies for the clades at different family level or above from DFP, DML, and DHB analyses were summarized (Table 3).

Table 3. Synapomorphies inferred from a democratic dataset of pollen characters for basal angiosperms analyzed with Fitch parsimony (DFP), maximum likelihood (DML), and hierarchical Bayesian inference (DHB). Pollen characters (†) and their states (§) are numbered in this table following Appendix 2. No synapomorphies were found for pollen character 10, aperature membrane. Superscripts refer to the number of the method used: [1]DFP, [2]DML, [3]DHB.

Taxa/Pollen character no.	1†	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Angiosperms			$1^{\S 3}$	1^{12}		0^{23}									3^{2}		3^{123}	
Annonaceae	1^{123}						3^{3}		1^{123}		2^{3}							
Aristolochiaceae		1^{3}						0^{3}			1^{3}							
Atherospermataceae														0^{1}				
Atherospermataceae + Gomortegaceae + Siparunaceae			0^{3}	0^{123}											7^{3}			
Austrobaileyales			0^{3}			3^{3}			4^{3}									
Austrobaileyales and all later-diverging angiosperms	2^{3}						2^{3}											
Cabombaceae			0^{123}	2^{3}														
Cabombaceae + Nymphaeaceae												1^{2}	1^{123}					
Calycanthaceae				2^{123}	2^{12}										1^{123}			
Canellaceae															0^{3}			
Canellales			0^{3}						1^{3}									
Ceratophyllales + basal eudicots + monocots				3^{3}														
Chloranthales				4^{2}								1^{2}	1^{123}					
Chloranthales + magnoliids				4^{3}														
Degeneriaceae + Myristicaceae							2^{3}											
Hernandiaceae														1^{1}				
Hernandiaceae + Lauraceae + Monimiaceae																0^{123}		0^{123}
Laurales		0^{23}						0^{23}			2^{3}				0^{2}			
Laurales + Magnoliales														1^{1}				
Laurales with Calycanthaceae excluded												1^{2}	1^{123}					
Magnoliaceae							2^{123}											
Magnoliales			0^{3}				2^{3}										1^{3}	
Magnoliales with Magnoliaceae excluded															0^{3}			
Nymphaeaceae																	2^{12}	
Nymphaeales																	2^{3}	
Piperaceae												1^{2}	1^{123}					
Piperaceae + Saururaceae							0^{123}								1^{23}			
Schisandraceae + Trimeniaceae								2^{12}										
Schisandraceae (with *Illicium* excluded)							0^{123}											
Winteraceae	1^{123}																	

In contrast to democratic maximum likelihood (DML), democratic Bayesian inference (DHB) generated a greater number of unambiguous character state changes at internal nodes for each of the 18 pollen characters, except for dispersal unit, aperture membrane, infratectum, and foot layer, for which all three methods generated an equal number of character state changes. Ten internal nodes were characterized by at least five characters that underwent unambiguous state change throughout the phylogeny (ingroup) with DHB. These nodes are the roots of the clades that comprise all sampled angiosperms, Nymphaeales, monocots, the clade comprising *Potamogeton* L. and *Triglochin* L., Chloranthales, the clade comprising *Aristolochia*, *Lactoris* Phil., and *Thottea* Rottb., Magnoliales, Annonaceae, Calycanthaceae, and Laurales. This last group displayed the most number of state changes, i.e., nine (Fig. 10).

Discussion

Systematic significance of pollen morphology in the basal angiosperms

In this study, synapomorphies or likely synapomorphies were inferred for various lineages of the

basal angiosperms for 17 out of the 18 pollen characters studied (the only exception being ornamentation of the aperture membrane). A comprehensively coded matrix was used with Fitch parsimony (CFP), and for all 18 pollen characters studied a democratically coded matrix was used, with three methods of ancestral state reconstruction, including Fitch parsimony (DFP), maximum likelihood (DML), and Bayesian inference (DHB). These results indicate that pollen morphology can play an important role in supporting systematic relationships among basal angiosperms. Characters such as pollen grain polarity and symmetry, aperture number, supratectal element presence, and tectum sculpture provided the highest number of synapomorphies for families or higher taxa within basal angiosperms and indicate their value in taxonomy and systematics. When tree topologies from the recent literature were compared (cf. Figs. 7–9), it was clear that relationships are not yet clearly determined among basal angiosperms based on molecular data. Nevertheless, the topology of Qiu et al. (2006, three genes) is the best supported by pollen morphologies. This topology yielded the greatest number of taxa supported by pollen synapomorphies (22) and likely synapomorphies (14) under CFP and was the most congruent with the palynological evidence. The number of synapomorphies obtained did not seem to depend upon the number of terminals in the topology in our study. The phylogenetic conclusions of Qiu et al. (2006, three genes) placed the Amborellaceae as sister to Nymphaeales and the Chloranthaceae as sister to *Ceratophyllum*. Both relationships are also correspondingly demonstrated by the most recent angiosperm reconstructions of Qiu et al. (2010), with four mitochondrial genes *atp1*, *matR*, *nad5*, and *rps3*, and Zhang et al. (2012), with five highly conserved, low-copy nuclear genes *SMC1*, *SMC2*, *MSH1*, *MLH1*, and *MCM5*.

In the following discussion, we present the palynological data and its systematic significance for each of the major lineages within basal angiosperms, and within the ANITA group (Amborellales + Nymphaeales + Austrobaileyales). It has become clear through many studies (including the 12 phylogenetic trees used here) that the Amborellales, Nymphaeales, and Austrobaileyales (sensu Angiosperm Phylogeny Group, 2009) represent the earliest diverging lineages of extant angiosperms. Several pollen synapomorphies exist to support a relationship for the Austrobaileyales with later-diverging angiosperms, such as isopolarity (by democratic Bayesian inference: DHB), radial symmetry (by comprehensive data with Fitch parsimony [CFP] trees 8, 10 and democratic maximum likelihood [DML]), pollen circular outline in polar view (by CFP trees 1, 2, 4 to 10, 12, and democratic Fitch parsimony [DFP]), inaperturate pollen (by CFP tree 10), and supratectal elements absence (by CFP trees 5, 12). Hereafter, we refer to tree numbers specified in Figures 7 to 9 after the analytical method. This hypothesis is also supported by three likely synapomorphies in the Fitch parsimony analysis: isopolarity by CFP trees 4, 6, radial symmetry by CFP tree 1, and supratectal elements as absent in CFP tree 10. This provides evidence for the more basal positions of the lineages that lead to the Amborellales and Nymphaeales. Recent phylogenetic studies have suggested two main arrangements for the Amborellales with respect to the Nymphaeales. Either the Amborellales lies sister to all other angiosperms, as indicated by parsimony phylogenetic analyses (Soltis et al., 1999; Mathews & Donoghue, 2000; Moore et al., 2007), or the Amborellales lies sister to the Nymphaeales and this pair then lies sister to the other angiosperms, as indicated by likelihood and Bayesian methods (Qiu et al., 2006; Soltis et al., 2007). Sampson (2000a) has suggested that the isolated position of Amborellales as sister group to all other living angiosperms is reinforced by its unique pollen exine sculpturing. We find one additional synapomorphy from pollen data (radial symmetry, supported by DHB) and one likely synapomorphy (reticulate tectum sculpture, supported by CFP tree 5) to support this basally branching position of the Amborellales, with the Nymphaeales grouping with all later-diverging angiosperms). However, a sister relationship between the Nymphaeales and Amborellales is exhibited in the most palynologically favored reconstruction of Qiu et al. (2006, three genes) as well as that of Qiu et al. (2010). This is supported by the two (albeit related) synapomorphies of the supratectal elements as present (CFP tree 8) and the supratectal element shape as spinulate (CFP trees 8, 11).

Within the Nymphaeales, supratectal elements spinulate (comprehensive data with Fitch parsimony [CFP] tree 7) support the monophyly of the Nymphaeaceae, separated from Cabombaceae as in the Angiosperm Phylogeny Group (2009). The monophyly of the Cabombaceae is supported by two pollen synapomorphies: (1) oblate shape (CFP trees 1, 4 to 9, 11, 12, democratic Fitch parsimony [DFP], maximum likelihood [DML], and democratic Bayesian inference [DHB]) and (2) large size (50–99 μm, DHB). The Austrobaileyales is supported by a single likely synapomorphy, supratectal elements absent (CFP tree 6), and the three synapomorphies of oblate shape, pollen with three or multiples of three apertures, and syncolpate apertures, as indicated by DHB. The

interfamilial relationships within this order are also supported by pollen synapomorphies. The Schisandraceae is supported by the two synapomorphies of radial symmetry, as indicated in CFP trees 5, 11, and three (or multiples of three) apertures, as shown in CFP trees 1, 4, 5, 8, 11. The clade of Schisandraceae and Trimeniaceae, leaving the Austrobaileyaceae at the base of the order, is supported by a single likely synapomorphy, radial pollen symmetry (CFP trees 1, 4, 6, 9, 12) and one synapomorphy, equatorial apertures (CFP trees 1, 4, 6, 9, 12).

Ceratophyllales. The position of Ceratophyllales has been highly unstable (Qiu et al., 2006), with five main alternative hypotheses: (1) as sister to the basal eudicots (Soltis et al., 1999; Müller et al., 2006); (2) nesting within monocots (Soltis et al., 1997); (3) as sister to monocots (Qiu et al., 1999); (4) as sister to Chloranthaceae (Qiu et al., 2005, 2006 eight genes, 2010; Zhang et al., 2012); or (5) as the most basally branching of angiosperms (Chase et al., 1993; Savolainen et al., 2000). Hickey and Taylor (1996) suggested that Ceratophyllales was a poor candidate for the basalmost position suggested by Chase et al. (1993), a study that did not include *Amborella* Baill. The clade comprising basal eudicots, Ceratophyllales, and monocots is supported by the pollen synapomorphies of small size (comprehensive data with Fitch parsimony [CFP] tree 6) and three apertures (democratic Bayesian inference [DHB]), and one likely synapomorphy, radial symmetry (CFP tree 6). The clade comprising monocots and Ceratophyllales is supported by one synapomorphy, apolarity (CFP trees 4, 6), whereas the clade comprising basal eudicots and Ceratophyllales is supported by an alternative synapomorphy, radial symmetry (CFP trees 5, 7; also a likely synapomorpy in trees 9, 11, 12). Although there is support for a close affinity between basal eudicots, Ceratophyllales, and monocots, the topology of Qiu et al. (2006), exhibiting a sister relationship between Ceratophyllales and Chloranthales, is best supported by pollen evidence among all six studied trees with similar taxa sampling (i.e., Qiu et al., 1999, 2000, 2005, 2006 three/eight genes; Soltis et al., 2011) and is consistent with the topologies from Qiu et al. (2010) and Zhang et al. (2012). Furthermore, Endress and Doyle (2009) revealed that the relationship of Ceratophyllales to Chloranthales is supported by five unequivocal floral synapomorphies, whereas its relationship to eudicots is supported by only one unequivocal morphological synapomorphy.

Chloranthales. The Chloranthales, composed of the single family Chloranthaceae, is an isolated lineage dating back to the Early Cretaceous period (Qiu et al., 2006). In our study, the Chloranthales clade is well supported by the three synapomorphies of isopolarity (comprehensive data with Fitch parsimony [CFP] trees 5, 11; democratic maximum likelihood [DML]), supratectal elements present (CFP tree 8; DML), and supratectal elements spinulate (CFP tree 8; DML, democratic Bayesian inference [DHB]), and one likely synapomorphy, radial symmetry (CFP trees 5, 6, 9, 11, 12). The Chloranthales has been placed in a variety of positions in different molecular studies (Jansen et al., 2007), which include its grouping with monocots (Müller et al., 2006), nesting within the Austrobaileyales (Hansen et al., 2007), or as sister to the Ceratophyllales (Duvall et al., 2006; Mathews, 2006; Qiu et al., 2006). The placement of this order remains one of the most difficult issues for resolving relationships among the deep nodes of the angiosperm tree of life (Hansen et al., 2007). However, the options for its placement have been reduced by recent studies such as those from Qiu et al. (2005, 2006, 2010), Moore et al. (2007), Soltis et al. (2007), and Zhang et al. (2012). Despite this controversy, the Chloranthales have been most recently assigned as sister to magnoliids in the Angiosperm Phylogeny Group (2009) and Soltis et al. (2011). Some pollen evidence can be found for the varying positions of this order. There is one likely synapomorphy radial symmetry (CFP tree 4) supporting the clade comprising Ceratophyllales, Chloranthales, and monocots, and another likely synapomorphy, isopolarity (CFP tree 1) for the clade comprising basal eudicots, Chloranthales, and magnoliids. Only multiaperturate pollen (DHB) supports the monophyletic grouping of Chloranthaceae and magnoliids as indicated by Soltis et al. (2011). The best supported relationship in this study is that of Ceratophyllales as sister to Chloranthales.

Magnoliids. Magnoliids have been identified as a large monophyletic group (Angiosperm Phylogeny Group, 2009), but affinities among their member orders (Magnoliales, Laurales, Piperales, and Canellales) need further study, especially because there is still a lack of morphological characters that define relationships among the included lineages (Qiu et al., 2005, 2006). The morphological cladistic analysis of Doyle and Endress (2000) did not identify any synapomorphies for magnoliids as a whole, although there are several morphological features that suggest links among the four orders (Kubitzki et al., 1993). In our analysis, multiple apertures were the only synapomorphy found for the magnoliids (democratic Bayesian inference [DHB]).

Pollen character states were found to define well the relationships of some orders and their subclades within the magnoliids. Pollen synapomorphies define

Magnoliales (oblate shape, DHB) and Laurales (apolarity, democratic maximum likelihood [DML], DHB; zonate aperture and supratectal elements spinulate, DHB). In more recent phylogenetic analyses, Magnoliales appear as sister to Laurales, and Piperales as sister to Canellales, with the exceptions including the analyses by Müller et al. (2006) and Soltis et al. (2007). Magnoliales and Laurales share few morphological synapomorphies (Qiu et al., 2000), but a pollen synapomorphy of the supratectal element size $\geq$ 0.5 μm (comprehensive data with Fitch parsimony [CFP] tree 12; DFP), and a likely synapomorphy of the imperforate tectal ornamentation (CFP trees 1, 3, 4) were inferred for their sister relationship. Porate apertures and oblate pollen shape were inferred as synapomorphies for Canellales (DHB), but no pollen synapomorphy was found either for Piperales or for the clade comprising Canellales and Piperales. Within Canellales, Winteraceae were defined by the two synapomorphies of dispersal as tetrads (CFP trees 1, 4 to 6, 8, 9, 11, 12; democratic Fitch parsimony [DFP], DML, DHB) and porate apertures (CFP trees 1, 4 to 6, 8, 9, 11, 12), and one likely synapomorphy, oblate pollen shape (CFP 1, 4 to 6, 8, 9, 11, 12; DFP). Pollen morphology may play an important role in the systematics of Laurales since up to eight taxa were inferred to be supported by pollen synapomorphies or likely synapomorphies using CFP, DFP, DML, and DHB. The pollen morphology of Calycanthaceae is very distinct from other members of the Laurales in being isopolar (CFP 3, 5, 8, 9, 11, 12), and having two apertures (CFP trees 1, 4, 6, 8; DFP, DML, DHB) and perforate tectum ornamentation (CFP trees 6, 12; DFP, DML, DHB), all three characters being likely synapomorphies or synapomorphies for this family. The sister relationship between Calycanthaceae and other members of Laurales is well supported by up to nine synapomorphies or likely synapomorphies, such as pollen apolarity (CFP trees 1, 4, 5, 8, 11, 12), radial symmetry (CFP trees 1, 4, 5, 11, 12), inaperturate pollen (CFP trees 1, 4, 5, 8, 11, 12), a zonate aperture (CFP trees 4 to 6, 8, 9, 11; DFP, DML), supratectal elements present (CFP tree 8), supratectal elements spinulate (CFP tree 8), an imperforate tectum ornamentation (CFP tree 12), infratectum absent (CFP tree 8), and foot layer absent (CFP tree 8). The monophyletic group comprising Monimiaceae, Lauraceae, and Hernandiaceae is well supported by morphological and molecular data (Renner et al., 2000). It is also strongly supported by pollen synapomorphies or likely synapomorphies, which include pollen apolarity (CFP trees 6, 9), radial symmetry (CFP trees 1, 6, 9), inaperturate pollen (CFP trees 6, 9), imperforate tectum sculpture (DHB), infratectum absent (CFP trees 1, 4, 6, 9, 11, 12; DFP, DML, DHB), and foot layer absent (CFP trees 1, 4, 6, 9, 11, 12; DFP, DML, DHB). The Atherospermataceae can be defined by one likely synapomorphy, pollen isopolarity (CFP trees 9, 12), and three synapomorphies: oblate shape (DML), supratectal elements with both size ranges of $<$ 0.5 μm and $\geq$ 0.5 μm evenly distributed across the grain (DFP), and tectum comprising rudimentary or partly fused hemispherical processes (CFP trees 1, 4, 6, 8, 9, 12; DFP, DML). In addition, we infer a few synapomorphies or likely synapomorphies that support other clade groupings within Laurales such as the Hernandiaceae, and a clade comprising Gomortegaceae, Hernandiaceae, Lauraceae, and Monimiaceae; a clade comprising Atherospermataceae and Siparunaceae; a clade comprising Atherospermataceae, Lauraceae, and Siparunaceae; and a clade comprising Atherospermataceae, Gomortegaceae, and Siparunaceae (Figs. 7–9).

Relationships within the Magnoliales are problematic, particularly with respect to the two alternative basal groups that have been proposed, Myristicaceae (Qiu et al., 1999, 2000, 2006; Soltis et al., 2000, 2007) or Magnoliaceae (Qiu et al., 2006; Soltis et al., 2011). Pollen characters slightly support the latter hypothesis, suggesting that the Magnoliaceae is distinctive within the Magnoliales, since all other members of this order share the synapomorphy of imperforate tectum ornamentation (comprehensive data with Fitch parsimony [CFP] tree 8; democratic Bayesian inference [DHB]; also a likely synapomorphy in CFP tree 12). Two likely synapomorphies (oblate pollen shape [CFP trees 1, 4, 6, 8, 9, 12] and proximal aperture [CFP trees 1, 4, 6, 8, 9, 12]) as well as the three synapomorphies (pollen dispersal as tetrads [democratic Fitch parsimony (DFP), democratic maximum likelihood (DML), DHB], very large grain size [DHB], and proximal aperture position [DFP, DML, DHB]) were found for the Annonaceae. One likely synapomorphy, granulate infratectum (CFP trees 8, 12), was found for the clade comprising Annonaceae, Eupomatiaceae, and Himantandraceae, providing weak support for this clade. The sister relationship between the Himantandraceae and Degeneriaceae is supported by the synapomorphy of the absence of a foot layer (CFP trees 1, 4, 5, 9, 11). The Piperaceae and Saururaceae are subtended by the synapomorphies supratectal elements present (CFP tree 8), spinulate pollen (CFP trees 8, 11), and boat-shaped pollen (CFP tree 3). The sister relationship between these two families as found in most recent molecular studies (Soltis et al., 2011) is

further supported by small-sized pollen (CFP tree 1, 3 to 9, 11, 12; DFP, DML, DHB).

Related lineages: Monocots and basal eudicots. Several different placements have been suggested for monocots, ranging from sister to all remaining angiosperms other than the ANITA group, part of a clade with magnoliids and Chloranthales, sister to magnoliids, sister to eudicots, or part of a clade with Ceratophyllales plus eudicots (see Moore et al. [2007] for a review). A few pollen character states, i.e., small size, three apertures, radial symmetry, and apolarity, as discussed under Ceratophyllales, support the affinity of monocots with basal eudicots and Ceratophyllaceae.

The natural lineage of eudicots (Qiu et al., 2005) is well supported by two pollen synapomorphies, pollen with three or more apertures (comprehensive data with Fitch parsimony [CFP] trees 1, 4, but a likely synapomorphy in trees 6, 7; and democratic maximum likelihood [DML]) and equatorially positioned apertures (CFP trees 1, 4, 6, 7), and two likely synapomorphies, isopolarity (CFP trees 7 to 9; a synapomorphy in DML) and radial symmetry (CFP tree 4). The eudicots have been broadly characterized by the possession of tricolpate (or tricolpate-derived) pollen (Doyle & Hotton, 1991; Furness, 2007), but this aperture type has evolved independently several times in angiosperms. However, the tricolpate structures in members of the ANITA group mainly follow Garside's rule (Garside, 1946; Erdtman, 1952), whereas that of the eudicots follows Fischer's rule (Erdtman, 1952; Furness & Rudall, 2004).

In our analysis, the absence of pollen supratectal elements is a likely synapomorphy (comprehensive data with Fitch parsimony [CFP] trees 2, 6) that supports the clade comprising basal eudicots, monocots, and Ceratophyllaceae as sister to magnoliids. *Nelumbo* Adans. has historically been included in the Nymphaeaceae, but in recent molecular phylogenies, the genus nests within basal eudicots (Hayes et al., 2000). Early studies of its seed and embryo led researchers to describe *Nelumbo* as dicotyledonous (Mirbel, 1809). Pollen morphology supports an isolated position of *Nelumbo* in the basal eudicots with some unique characters such as large grain size (ca. 45–70 μm), one, two, or multiple apertures, and a zonate ectoaperture shape for the pollen (Perveen, 1999; Banks et al., 2007).

POSSIBLE EVOLUTION OF POLLEN MORPHOLOGY IN BASAL ANGIOSPERMS

The plesiomorphic palynological condition for the angiosperms has not been ascertained yet, although it has been debated across several decades. Several studies, such as those from Walker and Doyle (1975), Walker (1976), Walker and Walker (1984), as well as Zavada and Crepet (1986), suggested that the primitive conditions for angiosperm pollen were distally monosulcate, large- to medium-sized, boat-shaped, smooth-walled, with a granular infratectum. Using paleobotanical data from Israel, Brenner (1996) considered that the most ancestral pollen was inaperturate with strongly reduced columellae, and that this syndrome was at least as old as the reputed ancestral monosulcate condition. Sampson (2000a) concluded that the first angiosperm pollen grains were probably monosulcate, with a distal aperture and a homogeneous or partly granular extexine, but without a tectate-columellate structure. Later, Doyle and Endress (2000) and Doyle (2005) inferred that the ancestral angiosperms had globose, monosulcate pollen with columellae and a thin endexine, rather than boat-shaped monosulcate pollen, with a granular infratectum and no endexine.

In the present study, plesiomorphic states for pollen morphology within the basal angiosperms are inferred to be dispersal as monads, and grains that are heteropolar, spheroidal, and mono-aperturate, with distal, colpate apertures having sculptured membranes, as well as supratectal elements with spinulate shape and size < 0.5 μm, with an infratectum and foot layer (based on comprehensive Fitch parsimony [CFP]). From democratic Fitch parsimony, maximum likelihood, and Bayesian inference (DFP, DML, and DHB, respectively), pollen grains are also inferred as bilateral, elliptic, with a tectate-rugulate surface and alveolate infratectum alveolate. We consider that the earliest pollen type of the basal angiosperms is, therefore, likely to be characterized by most of these pollen features.

The likely aperture condition of early angiosperms has been widely debated. Arguments for an inaperturate condition have mostly been based on observations of basally branching groups, e.g., Laurales, Magnoliales, and monocots. Brenner (1996) considered that the tetrads of Winteraceae evolved from monoporate monads, which had been derived from inaperturate pollen. In addition, he suggested that the evolution of apertures from the inaperturate condition gave a reproductive advantage. Hickey and Taylor (1996) corroborated Brenner's (1996) inference that angiosperm pollen was primitively spherical and inaperturate. Furthermore, the analyses of Qiu et al. (2006) and Soltis et al. (2008) reinforced the hypothesis that some of the earliest angiosperms may have been aquatic, given that aquatic or moist habitats may have provided a refuge from competition

with terrestrial gymnosperms during the early evolution of angiosperms, and inaperturate pollen is frequently found correlated with aquatic or moist habitats (as in monocots), where it may be an adaptation for submarine pollination (Ducker et al., 1978), and with functional dioecy in some lineages of eudicots (Furness, 2007).

The trichotomosulcate pollen type is another significant trend in the early evolution of the angiosperm pollen aperture. Trichotomosulcate pollen occurs in some Nymphaeales, the Chloranthaceae, and magnoliids, and has been recovered in fossilized waterlily-type flowers from the Eocene flora of the Messel oil shales (Harley, 2004). Zonasulcate pollen has also been proposed as an ancestral state in the evolution of pollen apertures. Zonasulcate pollen has been inferred to have evolved independently in several basal angiosperm families, including the Nymphaeales, Magnoliales, Laurales, and some monocots. The zonasulcate condition may in fact be derived from the trichotomosulcate condition in pollen (Harley & Dransfield, 2003). A ringlike, equatorially located aperture (i.e., zonate pollen) has been found in gymnosperm *Circumpolles* (Pflug, 1953; Klaus, 1960) fossils from as early as the Triassic and Jurassic periods (Pozhidaev, 2000; Hesse & Zetter, 2005).

Based on recent molecular data, monoaperturate paleoherbs (Donoghue & Doyle, 1989) are no longer considered to be the earliest diverging of the angiosperms (Angiosperm Phylogeny Group, 2009), and in contrast, the pollen of the earliest-diverging angiosperm, *Amborella*, has been found to contain a mixture of inaperturate grains and grains with a reduced apertural area (cf. Sampson, 1993). Similarly, based on our analyses, the ancestral state for pollen aperture type was inferred to be a monoaperture rather than inaperturate, as indicated by all methodologies (comprehensive data with Fitch parsimony [CFP], democratic Fitch parsimony [DFP], democratic maximum likelihood [DML], and democratic Bayesian inference [DHB]). This is more consistent with previous studies that suggested the monosulcate condition as the basal type for the earliest angiosperms on the basis of cladistic (e.g., Doyle & Donoghue, 1986; Doyle & Endress, 2000) and character optimization methods (e.g., Doyle, 2005).

In terms of pollen size, the first angiosperm pollen has variously been proposed as small (Macphail et al., 1999; Heimhofer et al., 2007) or medium to large (Walker & Doyle, 1975; Walker, 1976; Walker & Walker, 1984; Zavada & Crepet, 1986). Our results on 11 out of 12 studied phylogenies (with the exception of Mathews & Donoghue [2000]), using comprehensive data with Fitch parsimony (CFP), democratic maximum likelihood (DML), and democratic Bayesian inference (DHB), suggest that medium (25–49 μm, most commonly) or large size (50–99 μm) is inferred as the ancestral state for the basal angiosperms.

Regarding the tectum sculpture of pollen grains, Walker and Doyle (1975) suggested that ancestral angiosperm pollen was more or less psilate. Hughes and McDougall (1987) discovered that one of the oldest angiosperm-like monosulcate grains had verrucate sculpture elements covered with minute spinules very much as seen in the Amborellales (Sampson, 1993). Friis et al. (2006) suggested that early angiosperms were insect pollinated and had pollen with a reticulate surface. The reticulate tectum might be an adaptation to insect pollination (Zavialova & Gomankov, 2009). In our analysis, perforate-fossulate, rugulate, reticulate, and an *Amborella*-type ornamentation were all found to be potential ancestral states for tectum sculpture in angiosperms under comprehensive data with Fitch parsimony (CFP). Of these, rugulate ornamentation was inferred as ancestral on the greatest number of trees (CFP trees 2, 5, 11), being unambiguously inferred as the ancestral state by democratic Fitch parsimony, maximum likelihood, and Bayesian inference (DFP, DML, and DHB, respectively). Therefore, we consider that a rugulate tectum was the most likely plesiomorphic angiosperm type.

An alveolate infratectum was revealed as a potential plesiomorphy for angiosperms in our study, particularly on trees rooted with gymnosperm outgroups. However, further evidence from either paleopalynology or character evolution analysis seems to support the columellate type (Brenner, 1996; Doyle & Endress, 2000; Doyle, 2005). In most members of the ANITA group, the ectexine is tectate-columellate (Sampson, 2000a). Furthermore, Heimhofer et al. (2007) found that the columellate-tectate pollen grains of the †*Clavatipollenites* Couper 1958 group represent a common constituent of early angiosperm pollen assemblages. Additional study of this character would be worthwhile.

LIMITATIONS IN INFERRING PALYNOLOGICAL EVOLUTION IN EARLY DIVERGING ANGIOSPERMS

Uncertainty in molecular phylogenies. Although great progress has been made in genetic and genomic approaches to phylogenetics over past decades, the precise phylogenetic relationships of the basal angiosperms remain unclear. Complex evolutionary processes such as rapid radiation in terms of both

species and morphology, polyploid speciation, and convergent evolution may have played a substantial role in the very early divergence of the angiosperms, making the discernment of basal angiosperm phylogeny even more difficult. The explosive radiation of angiosperms appears to have begun with the origin of the mesangiosperm clade, after the origin of crown group angiosperms, and with the divergence of the more basal ANITA lines (Moore et al., 2007; Endress & Doyle, 2009). Many of the early-diverging major clades, such as the magnoliids, monocots, Chloranthaceae, eudicots, and Ceratophyllaceae, branched in rapid succession (Soltis et al., 2008). Polyploidy has long been recognized as a major evolutionary force in many plant lineages, in particular the ferns and angiosperms; cytology and recent genomic studies have also provided evidence for a polyploid origin of angiosperms (reviewed by Soltis et al., 2008). In addition, evolutionary parallelism is implicated in the evolution of basal angiosperms and some gymnosperms, inferred by the similarity in the features of double fertilization and flowers or flower-like structures between basal angiosperms and Gnetales (reviewed by Qiu et al., 2000). Thus, both methodological inadequacies and potentially intricate evolutionary patterns render a stable phylogenetic framework with robust support unavailable at the present time. Nonetheless, by exploring a number of possible topologies, as herein, we were able to draw certain significant conclusions when tracing pollen evolutionary trends in basal angiosperms.

Character convergence and functional constraints in palynological evolution. The occurrence of evolutionary parallelism or convergence in pollen morphology in the basal angiosperms has been detected both in our analyses and in previous studies (Le Thomas, 1980; Harley, 2004; Furness, 2007; Lora et al., 2009). Changes of state in pollen characters such as aperture type and polarity are already known to have occurred a number of times (Harley, 2004). While monosulcate or inaperturate pollen is traditionally considered to be plesiomorphic for angiosperms (Walker & Doyle, 1975; Brenner, 1996; Doyle, 2005), such pollen may represent interesting examples of convergent evolution (Furness, 2007). Evolution of inaperturate pollen, in particular, is sometimes associated with an aquatic habit, parasitism, insectivory, heterostyly, anemophily, or the presence of pollinia (Furness, 2007). Such physical conditions are known to greatly affect pollen morphology in angiosperms (Tekleva & Krassilov, 2009; Zavialova & Gomankov, 2009) and would include the early branching lineages.

Pollination mechanisms, in particular, may impose constraints leading to convergent evolution of pollen morphological syndromes. Entomophilous pollen is generally characterized by compound apertures, a large grain size, and a reticulate tectum; anemophilous pollen may be distinguished by simple apertures, spherical grain shape, small size, a better-developed exine, with a dry surface and scabrate-areolate tectum; and hydrophilous pollen may be inaperturate, less rigid, with a thin, elastic, and reduced exine, sometimes functionally filiform, and produced in large amounts (reviewed by Ackerman, 2000). Tectate-columellate pollen has been of functional significance for the action of harmomegathy (Sampson, 2000a). Genome size may determine or correlate with characters such as pollen size (Knight et al., 2010).

Some characters of angiosperm pollen are known to have appeared independently in gymnosperms much earlier than the putative origin of the angiosperms (Zavialova & Gomankov, 2009). As a result of adaptive radiation during the earliest angiosperm evolution (Endress, 1986), character convergence may confound our understanding of pollen evolutionary patterns and the plesiomorphic pollen type for angiosperms.

Ontogenic mode. Gabarayeva and Grigorjeva (2012) highlighted the crucial importance of ontogenetic data for interpreting the nature of the exine, especially the infratectum and endexine, in basal angiosperm pollen. They pointed out that past misinterpretation of the infratectum as granular in the Magnoliaceae (e.g., Walker, 1976) may be due to the absence of ontogenetic data. In mature grains, sporopollenin accumulation on the tectum, columellae, and foot layer is so extensive that the rather short columellae often look like granules, and the endexine is often completely obscured (Gabarayeva & Grigorjeva, 2012). If the fundamental details of pollen ontogeny are not taken into account, then pollen diversity and character evolution in the basal angiosperms may in some cases be misinterpreted.

As with other reproductive characters such as the nature of the endosperm (Floyd & Friedman, 2001), pollen morphologies (e.g., structure of apertures, exine, and polarity) may exhibit high variability in developmental mechanisms, particularly during the early radiation of the angiosperms (Heslop-Harrison & Heslop-Harrison, 1992; Ressayre et al., 2002; Furness & Rudall, 2004; Furness, 2007; Lora et al., 2009). Blackmore et al. (2007) reviewed self-assembly processes in pollen wall ontogeny of the flowering plants, which are not under direct genetic control but play an important role in pollen wall patterning.

According to this study, almost all eudicots exhibit simultaneous microsporogenesis, with exceptions including a few basal eudicots such as the Nelumbonaceae and Proteaceae. By contrast, in basal angiosperms, microsporogenesis and aperture pattern are highly labile and exhibit both the common patterns of simultaneous and successive microsporogenesis as well as intermediate types. Both monosulcate and inaperturate pollen can occur in association with either simultaneous or successive microsporogenesis (Blackmore & Crane, 1998; Furness & Rudall, 2004). Therefore, similar pollen characters may result from different pollen developmental modes.

For example, the orientation of colpi relative to the tetrad in the tricolpate or tricolpate-derived pollen of Austrobaileyales and other basal eudicots follows Garside's rule and is, therefore, different in origin to that of eudicots that follow Fischer's rule (reviewed by Furness & Rudall, 2004). Monosulcate pollen in the Austrobaileyaceae and Chloranthaceae may result from simultaneous cytokinesis, whereas in the Magnoliaceae monosulcate pollen may result from simultaneous cytokinesis or intermediate simultaneous cytokinesis, and in *Galbulimima* F. M. Bailey (Himantandraceae) from successive cytokinesis (Harley, 2004).

Incomplete fossil information. Due to the pivotal systematic position of the basal angiosperms among the seed plants, fossil data play a vital role in phylogenetic reconstruction and outgroup determination. In addition to rapid taxonomic radiation, extinctions among early angiosperm lineages greatly confound relationships among modern basal angiosperms. The living sister group to all angiosperms is still debated (Qiu et al., 2000; Endress & Doyle, 2009), and fossil relatives remain mysterious, although new insights from paleobotany continue to reduce the phylogenetic gap between extant gymnosperms and angiosperms (Qiu et al., 2000; Soltis et al., 2005; Endress & Doyle, 2009: Friis et al., 2011). Incorporating fossils of inferred close relationship into analyses of extant angiosperms may facilitate prediction of the morphological characters present in the early angiosperms, determination of plesiomorphies, and resolution of the early evolution of angiosperms (Hermsen et al., 2006). However, as with previous studies (Doyle & Endress, 2000; Doyle, 2005), analyses such as ours cannot, at present, make use of extinct taxa while based on molecular phylogenetic estimates.

Infraspecific polymorphism in pollen characters. In certain basal angiosperms, pollen polymorphism occurs within species, e.g., *Trimenia papuana* Ridl. (Trimeniaceae), which has dimorphic pollen, with both inaperturate and polyporate types (Sampson, 2007). Similarly, pollen grains of the basalmost angiosperm, *Amborella trichopoda*, have been observed as ana-ulcerate or anasulcate; however, Bailey and Swamy (1948), Erdtman (1952), Walker (1976), and Sampson (1993) found that a high proportion of its pollen examined under SEM showed no signs of apertures. Infraspecific pollen polymorphism, particularly in this species, may render interpretations of ancestral character states as equivocal in deep nodes and have a significant effect upon determination of plesiomorphic characters for angiosperms, especially because a close outgroup cannot be defined.

Incorrect or ambiguous pollen data from previous studies. The pollen data used for character optimization onto molecular phylogenies, in both our study (see Table 1) and those of Doyle and Endress (2000) and Doyle (2005), were drawn mainly from palynological reports in the literature made over past decades. While these data are very useful, they should be used with caution, particularly if conflicting observations are noted. For instance, the pollen infratectum of *Welwitschia* Hook. f. and *Gnetum* L. (both gymnosperm outgroups in our study) is described as columellate in Hickey and Taylor (1996) but as granular in Tekleva and Krassilov (2009). This could be explained either as a polymorphic character or as an incorrect description in one or both cases. Furthermore, pollen data for some taxa in the older literature made use of only light microscope (LM) observations. Although it is often incredible how much information can be gleaned from careful LM observation, some characters such as the internal foramina may only be visible under TEM electron microscopy. Gullvåg (1966) suggested that, in the Coniferales, electron microscopy should be used in order to check the detailed characteristics of pollen types, because the light microscope is sometimes deceptive. Therefore, previous SEM and TEM observations on pollen grains are very substantial in the study.

Physical morphological transformation. Pollen grains possess a variety of physiological and structural adaptations to dry environments and possible dehydration, such as harmomegathy. The most striking of these adaptations is the ability of the pollen wall to fold onto itself to prevent further desiccation (Katifori et al., 2010). Folding processes (e.g., taking up a "boat-shaped" outline) can be critical to the survival of the pollen grain. Temporary changes in functional pollen morphology have been detected in gymnosperms (Cycadales and Gink-

goales), basal angiosperms (including the Nymphaeaceae, Ceratophyllaceae, Eupomatiaceae, Annonaceae, Monimiaceae, and Atherospermataceae), and eudicots, such as *Limnanthes* R. Br., *Pedicularis* L., *Acer* L., and *Hypericum* L. (Clarke, 1975; Pozhidaev, 1993; Tang et al., 1995; Ackerman, 2000; Zhang et al., 2000; Hesse & Zetter, 2005; Tekleva et al., 2007). In these taxa, pollen structure may be situationally modified in adaptation to different environments. The degree of hydration also influences the actual pollen grain shape (Halbritter & Hesse, 2004). For instance, dehydrated pollen of some members of the Cycadales (e.g., *Cycas* L.) and Ginkgoales (e.g., *Ginkgo biloba* L.) appears boat-shaped, with a folded aperture region resembling a sulcus, whereas in the hydrated state, pollen grains are more or less spheroidal, and apparently the aperture occupies almost half of the pollen grain surface (Tang et al., 1995; Zhang et al., 2000; Tekleva et al., 2007).

In basal angiosperms, there are a number of potential waterproofing mechanisms acting on the pollen wall of hydrophilous plants such as the Ceratophyllaceae to prevent them from bursting. For example, these plants have omniaperturate pollen with a thin, elastic exine that accommodates some increase in grain size (Thanikaimoni, 1986). Physical changes in the zona-aperturate pollen of some early flowering plants in wet environments may represent functional benefits for possible pollen tube formation or pollen germination (Hesse & Zetter, 2005). Boat-shaped pollen with a single aperture is commonly found in some gymnosperms and basal angiosperms (Ginkgoaceae, Cycadaceae, Zamiaceae, Saururaceae, Magnoliaceae, Degeneriaceae, Myristicaceae, and Annonaceae), although the zona-aperturate type has also been considered as a possible plesiomorphic angiosperm aperture condition (Pozhidaev, 2000; Harley & Dransfield, 2003). It is important to determine whether these pollen shapes are permanent and stable or merely appear under certain environmental conditions.

Future Prospects

It is crucial to continue testing hypotheses of angiosperm origins and to answer questions about the nature of the basalmost lineages (Lockhart & Penny, 2005) in several ways. First, although plastid phylogenomic approaches to resolving the relationships of the basal angiosperms (Jansen et al., 2007; Moore et al., 2007; Angiosperm Phylogeny Group, 2009) now appear to provide a more robust topology, this should be confirmed using more nuclear sequence data (Soltis et al., 2008). Further phylogenetic work is necessary, especially to resolve the relationships among the Ceratophyllales, Chloranthales, monocots, magnoliids, and eudicots. This will provide not only a better understanding of the speciation, extinction, and rapid diversification that occurred among the early angiosperms, but also a solid framework for studies of morphological evolution. Second, new discoveries of early angiosperm fossil taxa and their integration into the phylogeny of angiosperms would provide further insights to evaluate the implications of fossils for phylogeny and the origin of pollen morphology. Third, advancing knowledge of pollen ontogeny (microsporogenesis) and physiology is crucial to the correct interpretation of homology in pollen morphological characters.

Nevertheless, by careful analysis of existing literature and new data, on the basis of multiple possible phylogenetic estimates and using multiple coding strategies and analysis methods, we have been able to conduct a detailed and integrated analysis of pollen morphology of the basal angiosperms. This study provides evidence of plesiomorphic characters in pollen, synapomorphies for extant taxa and monophyletic groups, and insights into evolutionary patterns, as well as a basis for further study of both the pollen evolution and systematics of this group.

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Appendix 1. Phylogenetic position and genus-level terminals used in the phylogenetic analyses. The generic totals for families are sensu Angiosperm Phylogeny Group (2009) and Soltis et al. (2011). Fifty-seven genus-level exemplars represent nine orders and 28 families of basal angiosperms. Ten genus-level exemplars span three orders and eight families of basal eudicots; three genus exemplars are included for two orders and three families of monocots. Eight outgroup genera represent four orders and eight families of gymnosperms.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
BASAL ANGIOSPERMS			
AMBORELLALES			
Amborellaceae	1 (1)	[2,3]*Amborella* Baill.*	Sampson, 1993, 2000a; Hesse, 2001; Suo, 2004
		[1]*Amborella trichopoda* Baill.	New Caledonia, *Nakada s.n.* (CANBRHB of deposit)
AUSTROBAILEYALES			
Austrobaileyaceae	1 (1)	[2,3]*Austrobaileya* C. T. White	Endress & Honegger, 1980; Zavada, 1984; Sampson, 2000a
		[1]*Austrobaileya scandens* C. T. White (Fig. 1G–I)	Australia, Queensland, *L. S. Smith 4631* (CANB)
Schisandraceae	3 (3)	[2,3]*Illicium* L.	Lin, 1989; Liu & Yang, 1989; Doyle et al., 1990; Takahashi, 1994; Sampson, 2000a; Gabarayeva & Grigorjeva, 2004; Wang et al., 2010
		[1]*Illicium macranthum* A. C. Sm. (Fig. 2C)	China, *Y. M. Shui et al. 002758* (KUN)
		[1]*Illicium micranthum* Dunn (Fig. 2A, B)	China, *L. Zhang ZL05* (HITBG)

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
		[2,3]*Kadsura* Juss.	Walker, 1974; Vijayaraghavan & Dhar, 1975; Praglowski, 1976; Lieux, 1980; Lan, 1984; Sampson, 2000a; Sun, 2000; Wang et al., 2010
		[2,3]*Schisandra* Michx.	Walker, 1974; Praglowski, 1976; Lieux, 1980; Sampson, 2000a; Sun, 2000; Wang et al., 2010
Trimeniaceae	1 (1)	[2,3]*Trimenia* Seem.	Sampson & Endress, 1984; Sampson, 1987, 2000a
		[1]*Trimenia moorei* (Oliv.) Philipson (Fig. 1J–L)	Australia, New South Wales, *P. H. Weston 2620* (E)
Canellales			
Canellaceae	6 (2)	[2,3]*Canella* P. Browne	Wilson, 1964; Sampson, 2000a
		[1]*Canella winterana* (L.) Gaertn. (Fig. 2G–I)	Dominican Republic, Barahona, *M. Fuertes 100* (E)
		[2,3]*Cinnamodendron* Endl.	Wilson, 1964; Sampson, 2000a
Winteraceae	5 (3)	[2,3]*Drimys* J. R. Forst. & G. Forst.*	Bailey & Nast, 1943; Lobreau-Callen, 1977; Praglowski, 1979; Sampson, 2000a; van der Ham & van Heuven, 2002
		[1]*Drimys piperita* Hook. f. (Fig. 2J–L)	Papua New Guinea, *J. R. Croft et al. LAE 65013* (E)
		[2,3]*Takhtajania* Baranova & J.-F. Leroy	Praglowski, 1979; Sampson, 2000a, 2000b; van der Ham & van Heuven, 2002
		[2,3]*Tasmannia* R. Br. ex DC.	Praglowski, 1979; van der Ham & van Heuven, 2002
Ceratophyllales			
Ceratophyllaceae	1 (1)	[2,3]*Ceratophyllum* L.	Díez et al., 1988; Takahashi, 1995
		[1]*Ceratophyllum* sp. indet. (Fig. 6J–L)	China, Gansu, *C. Schneider 1099* (E)
Chloranthales			
Chloranthaceae	4 (4)	[2,3]*Ascarina* J. R. Forst. & G. Forst.	Kuprianova, 1967; Todzia, 1988; Eklund et al., 2004
		[2,3]*Chloranthus* Sw.	Kuprianova, 1967; Todzia, 1988; Eklund et al., 2004
		[1]*Chloranthus holostegius* (Hand.-Mazz.) S. J. Pei & Shan (Fig. 2E, F)	China, Yunnan, *Germplasm Bank of Wild Species collection expedition 08CS162* (KUN)
		[1]*Chloranthus japonicus* Siebold (Fig. 2D)	China, Yunnan, *S. D. Zhang et al. APE015* (KUN)
		[2,3]*Hedyosmum* Sw.	Kuprianova, 1967; Todzia, 1988; Eklund et al., 2004
		[2,3]*Sarcandra* Gardner	Todzia, 1988; Eklund et al., 2004
Laurales			
Atherospermataceae	6 or 7 (3)	[2,3]*Atherosperma* Labill.	Sampson & Foreman, 1988
		[1]*Atherosperma moschatum* Labill. (Figs. 5L, 6A)	Australia, Victoria, *H. van Rees 048* (CANB)
		[2,3]*Daphnandra* Benth.	Sampson & Foreman, 1988; Sampson, 2000a
		[1]*Daphnandra tenuipes* G. Perkins (Fig. 6C)	Australia, New South Wales, *R. Schodde & H. C. Hayes 3572* (E)
		[2]*Doryphora* Endl.	Sampson & Foreman, 1988; Sampson, 2000a.
		[1]*Doryphora sassafras* Endl. (Fig. 6B)	Australia, New South Wales, *I. R. Telfora 1046* (CANB)
Calycanthaceae	4 (3)	[2,3]*Calycanthus* L.	Nicely, 1965; Li, 1990; Sampson, 2000a

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
		[1]*Calycanthus chinensis* (W. C. Cheng & S. Y. Chang) P. T. Li (Fig. 5D–F)	China, Zhejiang, *S. D. Zhang et al. APE026* (KUN)
		[2]*Chimonanthus* Lindl.*	Nicely, 1965; Sampson, 2000a
		[2,3]*Idiospermum* S. T. Blake	Sampson, 2000a; Staedler et al., 2009
Gomortegaceae	1 (1)	[2,3]*Gomortega* Ruiz & Pav.	Hesse & Kubitzki, 1983
		[1]*Gomortega keule* (Molina) Baill. (Fig. 5I–K)	Hesse & Kubitzki, 1983
Hernandiaceae	4 (2) (APG notes 5, but Kew notes 4)	[2,3]*Gyrocarpus* Jacq.	Sampson, 2000a
		[2,3]*Hernandia* L.	Hesse & Kubitzki, 1983; Sampson, 2000a
		[1]*Hernandia didymantha* Donn. Sm. (Fig. 6F, G)	Costa Rica, *B. Hammel 11656* (KUN)
Lauraceae	50 (3)	[2]*Cinnamomum* Schaeff.	Kubitzki, 1981; van der Merwe & van Wyk, 1990; Tang & Shang, 1995; Sampson, 2000a; Premathilake & Nilsson, 2001
		[2,3]*Cryptocarya* R. Br.	Kubitzki, 1981; Raj & van der Werff, 1988; van der Merwe & van Wyk, 1990; Rowley & Vasanthy, 1993; Tang & Shang, 1995; Sampson, 2000a
		[2,3]*Laurus* L.*	Kubitzki, 1981; van der Merwe & van Wyk, 1990; Tang & Shang, 1995; Sampson, 2000a
		[1]*Laurus nobilis* L. (Fig. 6D, E)	China, Yunnan, *K. M. Feng 24109* (KUN)
Monimiaceae	22 (2)	[2,3]*Hedycarya* J. R. Forst. & G. Forst.	Sampson, 1997; Sampson, 2000a; Doyle, 2005
		[2]*Hortonia* Wight & Arn.	Sampson, 1993, 2000a; Doyle, 2005
		[2,3]*Peumus* Molina	Sampson & Foreman, 1990; Sampson, 1993, 1997; Doyle, 2005
		[1]*Peumus boldus* Molina (Fig. 6H, I)	Chile, Valparaíso, *P. Hechenleitner Vega et al. 12* (E)
Siparunaceae	2 (1)	[2,3]*Siparuna* Aubl.	Pignal et al., 1999.
		[1]*Siparuna guianensis* Aubl. (Fig. 5G, H)	Brazil, Amazonas, *P. H. Davis & D. F. Coelho D. 60375* (E)
Magnoliales			
Annonaceae	129 (2)	[2,3]*Annona* L.	Walker, 1971a, 1971b; Le Thomas, 1980; Gottsberger & Silberbauer-Gottsberger, 1984; Doyle & Le Thomas, 1997; Sampson, 2000a
		[1]*Annona glabra* L. (Fig. 5A, B)	China, Yunnan, *L. Zhang ZL02* (HITBG)
		[1]*Annona muricata* L. (Fig. 4L)	China, Yunnan, *L. Zhang ZL01* (HITBG)
		[2,3]*Cananga* (DC.) Hook. f. & Thomson	Walker, 1971a; Le Thomas, 1980; Doyle & Le Thomas, 1997; Sampson, 2000a
		[1]*Cananga odorata* (Lam.) Hook. f. & Thomson var. *fruticosa* (Craib) J. Sinclair (Fig. 5C)	China, Yunnan, *L. Zhang ZL04* (HITBG)
Degeneriaceae	1 (1)	[2,3]*Degeneria* I. W. Bailey & A. C. Sm.	Dahl & Rowley, 1965; Sampson, 2000a
		[1]*Degeneria vitiensis* I. W. Bailey & A. C. Sm. (Fig. 4A–C)	Sampson, 2000a
Eupomatiaceae	1 (1)	[2,3]*Eupomatia* R. Br.	Hotchkiss, 1958; Woodland & Garlick, 1982; Sampson, 2000a
		[1]*Eupomatia laurina* R. Br. (Fig. 4I–K)	Australia, Queensland, Brisbane, 1875, *T. L. Bancroft s.n.* (E).
Himantandraceae	1 (1)	[2,3]*Galbulimima* F. M. Bailey	Sampson, 2000a

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
		[1]*Galbulimima baccata* F. M. Bailey (Fig. 4G, H)	Australia, Queensland, *PLF 27536* (CANB)
Magnoliaceae	2 (2)	[2,3]*Liriodendron* L.*	Canright, 1953; Agababian, 1972; Praglowski, 1974; Wei & Wu, 1993; Xu & Kirchoff, 2008
		[1]*Liriodendron tulipifera* L. (Fig. 3J)	China, cult., *Y. Z. Chu KUN 63-34* (KUN)
		[2,3]*Magnolia* L.	Canright, 1953; Agababian, 1972; Praglowski, 1974; Sampson, 2000a; Xu & Kirchoff, 2008
		[1]*Magnolia grandiflora* L. (Fig. 3K, L)	China, cult., *Y. T. Liu 5058* (KUN)
Myristicaceae	20 (3)	[2]*Knema* Lour.	Nair, 1965; Sauquet & Le Thomas, 2003; Sauquet et al., 2003
		[1]*Knema globularia* (Lam.) Warb. (Fig. 4D, E)	China, Yunnan, *Y. H. Li 005098* (KUN)
		[2,3]*Maloutchia* Warb.	Walker & Walker, 1981; Sauquet & Le Thomas, 2003; Sauquet et al., 2003
		[2,3]*Myristica* Gronov.	Nair, 1965; Sauquet & Le Thomas, 2003; Sauquet et al., 2003
		[1]*Myristica*, sp. indet. (Fig. 4F)	Papua New Guinea, Milne Bay, *J. R. Croft et al. LAE 71132* (E)
NYMPHAEALES			
Hydatellaceae	1 (1)	[2,3]*Trithuria* Hook. f.	Remizowa et al., 2008
		[1]*Trithuria australis* (Diels) D. D. Sokoloff, Remizowa, T. D. Macfarl. & Rudall (Fig. 1C)	Australia, Western Australia, *T. D. Macfarlane & A. R. Annels 2283* (PERTH)
Cabombaceae	2 (2)	[2,3]*Brasenia* Schreb.	Osborn et al., 1991; Taylor & Osborn, 2006
		[2,3]*Cabomba* Aubl.*	Osborn et al., 1991; Taylor et al., 2008
Nymphaeaceae	6 (2)	[2,3]*Nuphar* Sm.*	Meyer, 1964, 1966; Sampson, 2000a; Taylor & Osborn, 2006
		[1]*Nuphar luteum* Walp. (Fig. 1F)	U.S.A., Utech, *W. W. Thomas 82–530* (KUN)
		[2,3]*Nymphaea* L.*	Meyer, 1964, 1966; Gabarayeva & El-Ghazaly, 1997; Perveen, 1999; Sampson, 2000a; Gabarayeva et al., 2001; Taylor & Osborn, 2006
		[1]*Nymphaea alba* L. (Fig. 1D, E)	China, cult. from Germany, *S. D. Zhang et al. APE021* (KUN)
PIPERALES			
Aristolochiaceae	5 to 8 (5)	[2,3]*Aristolochia* L.*	Nair, 1965; Sampson, 2000a; González et al., 2001; Mulder, 2003; Perveen & Qaiser, 2008
		[1]*Aristolochia contorta* Bunge (Fig. 3E, F)	China, cult., *S. D. Zhang APE016* (KUN)
		[2]*Asarum* L.*	Mi & Yang, 1991; Sampson, 2000a; González et al., 2001; Mulder, 2003
		[2,3]*Lactoris* Phil.	Zavada & Taylor, 1986; Sampson, 1995, 2000a
		[1]*Lactoris fernandeziana* Phil. (Fig. 3B–D)	Zavada & Taylor, 1986.
		[2,3]*Saruma* Oliv.	Dickison, 1992; Sampson, 2000a; González et al., 2001
		[2,3]*Thottea* Rottb.	González et al., 2001
Piperaceae	5 (2)	[2,3]*Peperomia* Ruiz & Pav.*	Nair, 1965; Lei & Liang, 1998; Sampson, 2000a

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
		[1]*Peperomia heyneana* Miq. (Fig. 3G)	China, Yunnan, *Y. L. Li et al. YDDXS0406* (KUN)
		[2,3]*Piper* L.*	Nair, 1965; Huang & Huang, 1997; Lei & Liang, 1998; Sampson, 2000a; Suwanphakdee et al., 2008
Hydnoraceae	2 (1)	[1]*Hydnora* Thun.	original description
		[1]*Hydnora abyssinica* A. Braun (Fig. 3A)	Sudan, Blue Nile at Um Barona, near Wad Medani, *L. J. Musselman 198/6129* (E)
Saururaceae	5 (3)	[2]*Anemopsis* Hook. & Arn.	Xi, 1980; Liang, 1992; Smith & Stockey, 2007
		[2,3]*Houttuynia* Thunb.	Xi, 1980; Takahashi, 1986; Liang, 1992; Smith & Stockey, 2007
		[1]*Houttuynia cordata* Thunb. (Fig. 3I)	China, Yunnan, *Germplasm Bank of Wild Species collection expedition 09CS1221* (KUN)
		[2,3]*Saururus* L.	Xi, 1980; Liang, 1992; Sampson, 2000a; Smith & Stockey, 2007
		[1]*Saururus chinensis* (Lour.) Baill. (Fig. 3H)	China, Guangxi, cult., *S. D. Zhang APE011* (KUN)
Basal eudicots			
Proteales			
Nelumbonaceae	1 (1)	[2,3]*Nelumbo* Adans.*	Kuprianova & Tarasevich, 1983; Blackmore et al., 1995; Kreunen & Osborn, 1999; Perveen, 1999; Banks et al., 2007.
Sabiaceae	3 (2)	[2,3]*Sabia* Colebr.	Furness et al., 2007
		[2,3]*Meliosma* Blume	Furness et al., 2007
Trochodendrales			
Trochodendraceae	2 (2)	[2,3]*Tetracentron* Oliv.	Praglowski, 1975; Wang et al., 1984; Zavada & Dilcher, 1986
		[2,3]*Trochodendron* Siebold & Zucc.	Praglowski, 1975; Wang et al., 1984; Zavada & Dilcher, 1986
Ranunculales			
Berberidaceae	15 (1)	[2]*Mahonia* Nutt.*	Nowicke & Skvarla, 1981; Blackmore & Heath, 1984
Menispermaceae	70 (1)	[2]*Cissampelos* L.	Harley & Ferguson, 1982
Lardizabalaceae	8 or 9 (1)	[2]*Akebia* Decne.*	Blackmore et al., 1995; Wei & Li, 1995
Papaveraceae	20 (1)	[2,3]*Dicentra* Bernh.*	Stern, 1962; Blackmore et al., 1995
Eupteleaceae	1 (1)	[2,3]*Euptelea* Siebold & Zucc.	Praglowski, 1975; Wang et al., 1984; Zavada & Dilcher, 1986
Monocots			
Alismatales			
Potamogetonaceae	6 (1)	[2,3]*Potamogeton* L.*	Wang, 1990; Perveen, 1999
Juncaginaceae	4 (1)	[2,3]*Triglochin* L.*	Wang, 1990
Acorales			
Acoraceae	1 (1)	[2,3]*Acorus* L.	Rudall & Furness, 1997; Wang & Zhao, 2002
Gymnosperm outgroups			
Gnetales			
Gnetaceae	1 (1)	[2,3]*Gnetum* L.	Gullvåg, 1966; Yao et al., 2004
Welwitschiaceae	1 (1)	[2,3]*Welwitschia* Hook. f.*	Gullvåg, 1966; Rydin & Friis, 2005
Pinales			
Pinaceae	11 (1)	[2,3]*Pinus* L.*	Pacini et al., 1999
Cupressaceae	30 (1)	[2,3]*Metasequoia* Hu & W. C. Cheng	Xi, 1988; Xi & Wang, 1989; Liu & Basinger, 2009
Podocarpaceae	16 (1)	[2,3]*Podocarpus* L'Hér. ex Pers.	Del Fueyo, 1996

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
CYCADALES			
Zamiaceae	9 or 10 (1)	[2,3]*Zamia* L.	Zavada, 1983; Dehgan & Dehgan, 1988; Tekleva et al., 2007.
Cycadaceae	1 (1)	[2,3]*Cycas* L.	Tang et al., 1995; Tekleva et al., 2007
GINKGOALES			
Ginkgoaceae	1 (1)	[2,3]*Ginkgo* L.*	Pacini et al., 1999; Zhang et al., 2000; Tekleva et al., 2007

* Additional data were taken from PalDat – Palynological Database, an Online Publication on fossil and recent Pollen and Spores, at <http://www.paldat.org/>.

[1] A representative species was selected to show pollen morphological diversity (cf. Figs. 1–6).

[2] The genus is represented in analyses using the comprehensive method with Fitch parsimony (CFP) on 12 different phylogenetic trees (Table 1 and Figs. 7–9).

[3] The genus is represented in analyses using the democratic method with Fitch parsimony (DFP), maximum likelihood (DML), and hierarchical Bayesian inference (DHB) on the maximum likelihood tree based on data from Soltis et al., 2011; Tables 2, 3, and Fig. 10).

APPENDIX 2. Pollen characters and their states as defined in this study.

1. Dispersal unit: 0, monads; 1, tetrads.
2. Polarity: 0, apolar; 1, heteropolar; 2, isopolar (or subisopolar).
3. Symmetry (in polar view): 0, bilateral; 1, radial.
4. Basic shape: 0, boat-shaped; 1, globose.
5. Shape class: 0, oblate; 1, spheroidal; 2, prolate.
6. Outline in polar view (amb): 0, circular; 1, elliptic.
7. Size (diameter of largest axis), states defined by Walker and Doyle (1975): 0, small (10–24 μm); 1, medium (25–49 μm); 2, large (50–99 μm); 3, very large (100–199 μm); 4, gigantic (> 200 μm).
8. Aperture number: 0, zero; 1, one; 2, two; 3, three or multiples of three; 4, more than three.
9. Aperture position: 0, polar: distal; 1, polar: proximal; 2, equatorial; 3, global.
10. Aperture membrane: 0, smooth; 1, sculptured.
11. Ectoaperture shape: 0, colpate; 1, porate; 2, zonate; 3, spiral; 4, syncolpate.
12. Supratectal element: 0, absent; 1, present.
13. Supratectal element shape: 0, verrucate; 1, spinulate.
14. Supratectal element size: 0, < 0.5 μm; 1, ≥ 0.5 μm.
15. Tectum sculpture: 0, imperforate (psilate); 1, perforate/fossulate (micro- and macro-); 2, rugulate; 3, reticulate (including striato-reticulate); 4, areolate; 5, striate; 6, *Amborella*-type ("a gcmmate [*sic*]-like appearance but consisting of small cupules constructed of coiled cylindrical strands"), described by Sampson (1993: 1); 7, rudimentary, i.e., partly fused hemispherical processes supported by columellae, terminology from Sampson and Foreman (1988).
16. Infratectum: 0, absent; 1, present.
17. Infratectum structure: 0, alveolate; 1, granulate; 2, intermediate (between granulate and columellate), terminology from Doyle (2005); 3, columellate.
18. Foot layer: 0, absent; 1, present.

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Cover illustration. *Randia truncata* Greenm. & Thomps., taken from J. H. Greenman & C. H. Thompson. 1914. Diagnoses of flowering plants, chiefly from southwestern United States and Mexico. Ann. Missouri Bot. Gard. 1(4): 405–418. This image commemorates the centennial of *Annals of the Missouri Botanical Garden*.

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EVOLUTIONARY TRENDS AND SPECIALIZATION IN THE EUGLOSSINE BEE–POLLINATED ORCHID GENUS *GONGORA*[1,2]

Molly C. Hetherington-Rauth[3] *and Santiago R. Ramírez*[3,4]

ABSTRACT

The family Orchidaceae exhibits some of the most diverse and intricate modes of animal pollination across angiosperms. Highly specialized pollination by male euglossine bees (Apidae, Euglossini) occurs in more than 600 species of Neotropical orchids. Male euglossine bees acquire volatile compounds from both floral and nonfloral sources, which they store in their specialized hind tibiae and later expose during courtship display. Euglossine-pollinated Orchidaceae produce large quantities of floral scent, which serves as both the attractant and reward for male euglossine bees. Upon collecting floral volatiles and aided by the intricate orchid floral morphology, male bees remove and subsequently deposit orchid pollinaria, resulting in pollination. Among euglossine-pollinated Orchidaceae is the species-rich genus *Gongora* Ruiz & Pav., which provides exceptional opportunities to investigate the evolution of scent-mediated pollinator specialization. Here we review the taxonomy, systematics, and pollination biology of *Gongora*. We also describe a new physical mechanism of pollination observed for *Gongora* and discuss the significance of different modes of pollinaria attachment in an evolutionary framework. This work provides the foundation for future research on the evolution of specialized plant–pollinator mutualisms, including elucidating the evolutionary relationships of cryptic species, understanding the evolution of floral adaptations, and investigating the mechanisms of speciation.

Key words: *Eufriesea*, *Euglossa*, euglossine bees, *Eulaema*, evolution, *Exaerete*, floral adaptations, *Gongora*, mutualism, Orchidaceae, pollination, speciation.

The Orchidaceae, with their staggering species diversity and uniquely specialized and intricate pollination mechanisms, have long attracted the interest of biologists and naturalists (Darwin, 1888; Dressler, 1981; Schiestl & Schlüter, 2009; Yam et al., 2009). Darwin remarked in his book devoted to the topic that "the contrivances by which Orchids are fertilised, are as varied and almost as perfect as any of the most beautiful adaptations in the animal kingdom" (Darwin, 1888: 1). These "contrivances," or mechanisms by which orchids are fertilized, are epitomized by the male euglossine bee–pollinated

[1] This and the following three articles are the proceedings of the 61st Annual Systematics Symposium of the Missouri Botanical Garden, "Integrating Approaches to Macroevolution: Combining Fossils and Phylogeny." The symposium was held 10–11 October 2014, at the Missouri Botanical Garden in St. Louis, Missouri, U.S.A.

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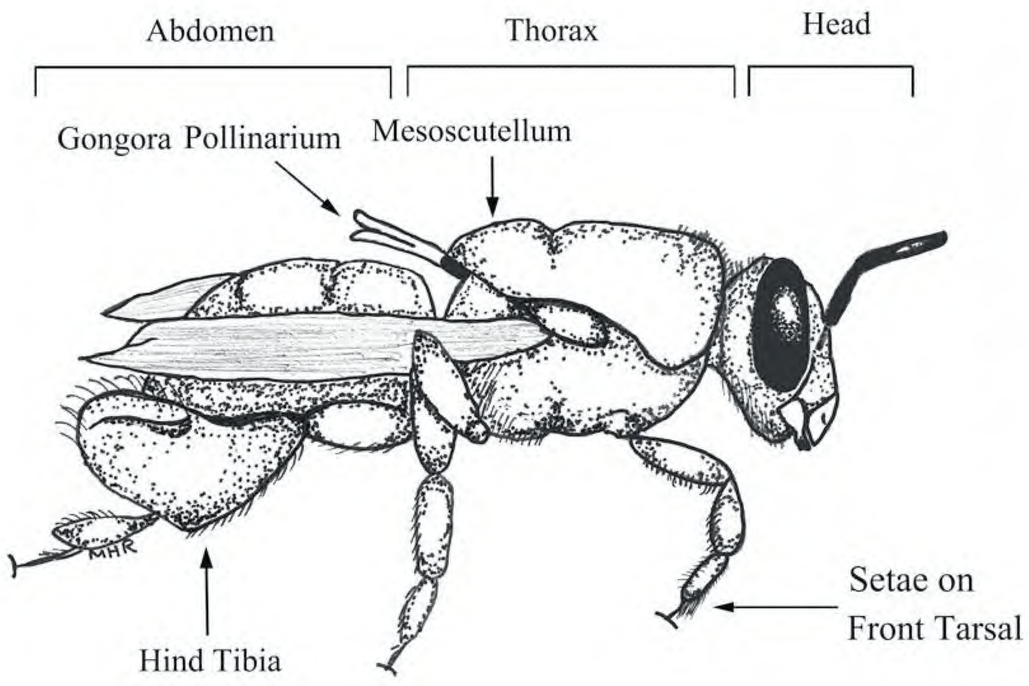


Figure 1. A generalized line drawing of a male euglossine bee of the genus *Euglossa* Latreille, with selected anatomical features important for the pollination of *Gongora* Ruiz & Pav. labeled. As depicted, the *Gongora* pollinarium is attached to the underside of the meoscutellum.

Orchidaceae, which include more than 600 species, or 10%, of Neotropical orchids (Gerlach & Schill, 1991; Ramírez et al., 2011).

Male euglossine bee pollination refers to the exclusive and specialized pollination of scent-producing orchids by male euglossine bees (also referred to as orchid bees) seeking perfume compounds (Dressler, 1968b; Dodson et al., 1969; Roubik & Hanson, 2004). There are more than 230 described species of euglossine bees (Apidae, Euglossini) grouped in five extant genera: *Aglae* Lepeletier & Serville, *Eufriesea* Crockerell, *Euglossa* Latreille, *Eulaema* Lepeletier, and *Exaerete* Hoffmannsegg (Nemésio & Rasmussen, 2011). The last four of these euglossine bee genera are abbreviated hereafter as *Ef.*, *Eg.*, *El.*, and *Ex.*, respectively. Euglossine bees exhibit brightly colored integument, ranging from blue and green to red and bronze, and are characterized by their unique behavior of scent collection. Males do not produce their own pheromones and instead collect volatile compounds from both floral (including orchids and several other angiosperm families such as Araceae, Solanaceae, and Gesneriaceae) and nonfloral sources (including resins, fungi, and rotting vegetation) in order to concoct species-specific perfume blends (Dressler, 1982a; Whitten et al., 1993; Eltz et al., 1999; Ramírez et al., 2002; Eltz, 2010). Perfumes are stored in specialized enlarged pockets located in the hind tibiae (Fig. 1) (Cruz-Landim et al., 1965; Eltz et al., 2007). Once male bees accumulate sufficient quantities of perfume compounds, they perform an elaborate courtship display in which perfumes are dispersed and exposed to conspecific females (Eltz et al., 1999, 2003, 2005). These chemical signals are hypothesized to convey information to females on male quality (fitness) and/or identity (species), and thus likely play a critical role in mate recognition and reproductive isolation (Zimmermann et al., 2009).

The pollination of orchids by euglossine bees was first described by Herman Crüger, the director of the Botanical Garden, Trinidad, in 1864 and later echoed by Darwin in his book discussing the pollination of orchids (Crüger, 1864; Darwin, 1888). Unsure of the exact mechanism, Crüger and Darwin described the "humble bees" as "gnawing" at the labellum (a highly modified petal) despite noting that the flower did not produce any nectar, which would otherwise explain the bee's behavior (Crüger, 1864: 129; Darwin, 1888: 168). Later in 1901, Adolpho Ducke, an entomologist and botanist studying in the Amazon, noted that several genera of orchids were visited by only male euglossine bees, but he too believed that the bees were visiting the flowers in search of food (Ducke, 1901; Dressler, 1968b; Dodson et al., 1969). It was not until the 1960s and through the extensive field observations made by Dodson, Frymire, Dressler, and Vogel that the odd behavior of perfume collection by male euglossines and the particular mechanism of euglossine bee pollination were elucidated (Dodson & Frymire, 1961; Vogel, 1963a, 1963b, 1966, 1990; Dodson, 1965; Dressler, 1968a; Dodson et al., 1969).

Male euglossine bee–pollinated Orchidaceae do not produce floral rewards in the form of nectar or pollen nor are they deceptive like many other orchid taxa that mimic food-rewarding plants or female bees (pseudocopulation) as deceptive mechanisms for pollinator attraction. Rather, male euglossine bee–pollinated orchids produce large quantities of floral scent, which simultaneously acts as the floral attractant and floral reward (Dodson & Frymire, 1961; Vogel, 1963a, 1990; Dodson et al., 1969). Male bees visit orchids to collect volatile compounds, and in the process they pollinate the flowers, aided almost entirely by the orchid's intricate floral morphology, which positions the pollinator in the proper, but often awkward, position. This awkward position ensures the precise placement of the orchid's pollinarium (consisting of the pollinia or pollen packets and the associated structures) on the bee's body, which in turn later ensures successful deposition of the pollinia into the stigma (Dressler, 1981).

Euglossine orchids tend to exhibit highly specialized pollinator associations. Pollinator specificity plays a significant role in mediating the extent of gene flow between populations, in maintaining reproductive isolating barriers, and in minimizing pollen wastage. In euglossine-pollinated Orchidaceae, pollinator identity is governed by both floral scent and floral morphology. The chemical composition of the floral

scent of euglossine-pollinated Orchidaceae tends to be dominated by volatile monoterpenoids, sesquiterpenoids, and aromatic compounds (Williams & Whitten, 1983). Each orchid species produces a species-specific floral scent, which usually consists of one to three compounds in large quantities and an additional one to 10 compounds in smaller amounts (Hills et al., 1972; Gregg, 1983; Whitten, 1985; Williams & Whitten, 1999). It is hypothesized that some of these compounds act as attractants, luring many species of male bees, whereas others serve as behavioral modifiers, selectively excluding species of male euglossine bees (Dodson et al., 1969; Dodson, 1970; Williams & Dodson, 1972). In this way, each orchid species is visited by only one or a few species of male euglossine bees. In addition, the floral morphology of the orchid serves to further increase pollinator specificity. Only a subset of the bees attracted to the floral scent will be of the correct size and exhibit the right behavior to serve as effective pollinators. In some instances, the visitor may be too large or too small to successfully remove and then deposit the pollinia (Dressler, 1968a; Hills et al., 1972).

Male euglossine bee pollination has evolved at least three times independently, resulting in the three separate orchid radiations in the Stanhopeinae + Coeliopsidinae, Zygopetalinae, and Catasetinae, all of which are placed within the tribe Cymbidieae (previously referred to as Maxillarieae) (Whitten et al., 2000, 2005; Ramírez et al., 2002, 2011; Chase et al., 2003, 2015). Additional independent origins of euglossine bee pollination are evident in the tribe Cymbidieae; however, these origins have not resulted in large radiation events (Ramírez et al., 2002; Chase et al., 2003, 2015; Neubig et al., 2012). A fossil-calibrated molecular clock analysis placed the origin of euglossine pollination for each of the three orchid lineages during the Oligocene–Miocene time period, ca. 18–27 million years ago (Ma), and the single origin of perfume collection behavior in male euglossine bees during the Eocene–Oligocene time period, ca. 34–38 Ma (Ramírez et al., 2011). These estimates therefore suggest that euglossine pollination in the Orchidaceae evolved much later than the origin of perfume-collecting behavior of male euglossine bees. Furthermore, comparing the rates of diversification between the orchid and bee lineages shows that although the diversification rate of male euglossine bee–pollinated orchids has increased toward the present, the diversification rate of euglossine bees has decreased toward the present (Ramírez et al., 2011). These patterns, along with the observation that male euglossine bees collect volatile compounds from non-orchid sources, lend support to the hypothesis of asynchronous diversification and asymmetric dependency in this specialized plant-pollinator mutualism and suggest that the pre-existing behavior of male euglossine bees drove the diversification of euglossine bee–pollinated Orchidaceae (Ramírez et al., 2011).

Systematics of the Genus *Gongora*

The euglossine bee–pollinated orchid genus *Gongora* Ruiz & Pav. (Stanhopeinae) has been estimated to have diverged from the rest of the Stanhopeinae at least 15 million years ago (Ramírez et al., 2011). Although the age estimate of a most recent common ancestor is sensitive to taxon sampling, the age of this node is unlikely to change given the high support for the monophyly of genera within the Stanhopeinae. *Gongora* is broadly distributed throughout the Neotropical region, with a range that extends from southern Mexico into South America along the slopes of the Andes from Colombia to Peru (and possibly Bolivia) and in areas of Venezuela, the Guianas, and Brazil (Fig. 2A) (Jenny, 1993). *Gongora* orchids are long-lived perennial epiphytes often found growing on the bark of trees. The vegetative portion consists of several conical to ovoid pseudobulbs with pronounced vertical ridges connected by a short rhizome and usually two broadly lanceolate-shaped leaves per pseudobulb (Fig. 3A–F). From the base of the pseudobulb emerges a spike that develops into a pendent inflorescence, which in some species can reach up to a meter long and contain over 50 individual flowers. The number of flowers per inflorescence may vary within species depending on plant health; most species tend to produce inflorescences with 10 to 20 flowers (Hetherington-Rauth, pers. obs.). Flowers typically bloom for three to seven days before wilting and typically produce a fragrance that is strongest in the morning, corresponding to the time of day when male euglossine bees are most active (Dressler, 1968a; Hills, 1989; Hills & Williams, 1990; Hetherington-Rauth, pers. obs.). The individual flowers are composed of two lateral sepals, one lower sepal, two small petals, a highly modified petal called the labellum, and the column, which consists of both the male and female reproductive structures (Fig. 3E) (Dressler, 1981; Whitten, 1985).

Gongora currently contains between 60 and 70 recognized species; however, many species are poorly known from only a few specimens (Jenny, 1993; Aldrich & Higgins, 2008; The Plant List, 2013). The taxonomic delimitation and systematics of most taxa within *Gongora* are notoriously difficult due to intraspecific variation and the lack of conspicuous diagnostic morphological characters for many species (Dressler, 1966; Jenny, 1993). This taxonomic difficulty has resulted in significant historical and present-

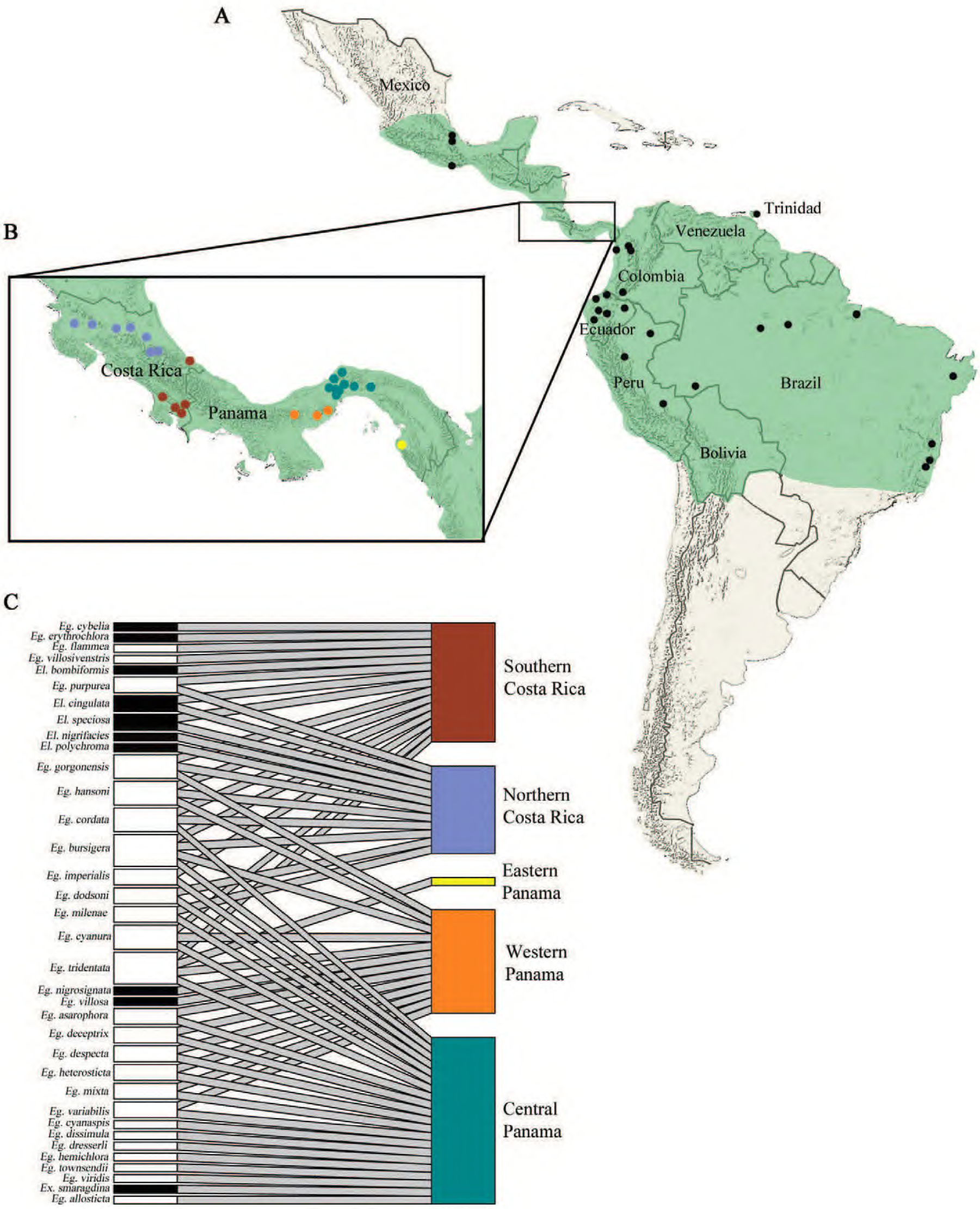


Figure 2. Geographic distribution of the orchid genus *Gongora* Ruiz & Pav. and pollination network. —A. Geographic distribution of *Gongora* (green shading) as inferred from Jenny (1993). Points within this geographic range indicate the localities where orchid-pollinator associations for *Gongora* have been observed (cf. Appendix 1). —B. Enlarged region of Costa Rica and Panama from A. The points again indicate the localities where orchid-pollinator associations for *Gongora* have been observed (cf. Appendix 1). The colors of the points correspond to the five geographic regions from the pollination network in C. —C. Bipartite pollination network depicting the pollinator diversity and pollinator specialization across five geographic regions in Costa Rica and Panama. The nodes (boxes) to the left represent species of euglossine bee visitors and pollinators. Gray boxes represent species of bee that were identified as actual pollinators (either observed pollinating *Gongora* [1] or collected with *Gongora* pollinaria [3]). Black boxes represent species of bee that are identified as non-pollinating visitors (2) or visitors with unknown pollination effect (4). Abbreviations used for the three euglossine bee genera are *Euglossa* (*Eg.*), *Eulaema* (*El.*), and *Exerate* (*Ex.*). The nodes (boxes) to the right represent *Gongora* that have been geographically clustered into five separate geographic regions such that each node likely includes co-occurring species of *Gongora*. The lines connecting nodes represent pollination observations between *Gongora* within the given geographic region and the indicated bee species.

day confusion in the application of species names within *Gongora*. The taxonomic difficulty of this group goes back to the times of the Royal Botanical Expedition to New Granada. During the last part of the 18th century, the botanists Ruiz and Pavón were commissioned by the Spanish viceroy, Charles III, to conduct a scientific exploration of the flora of what is now Peru and Chile (Jenny, 1996; Pupulin, 2012). Among the flora they collected that was not captured, lost, or destroyed was what was to become the infamous

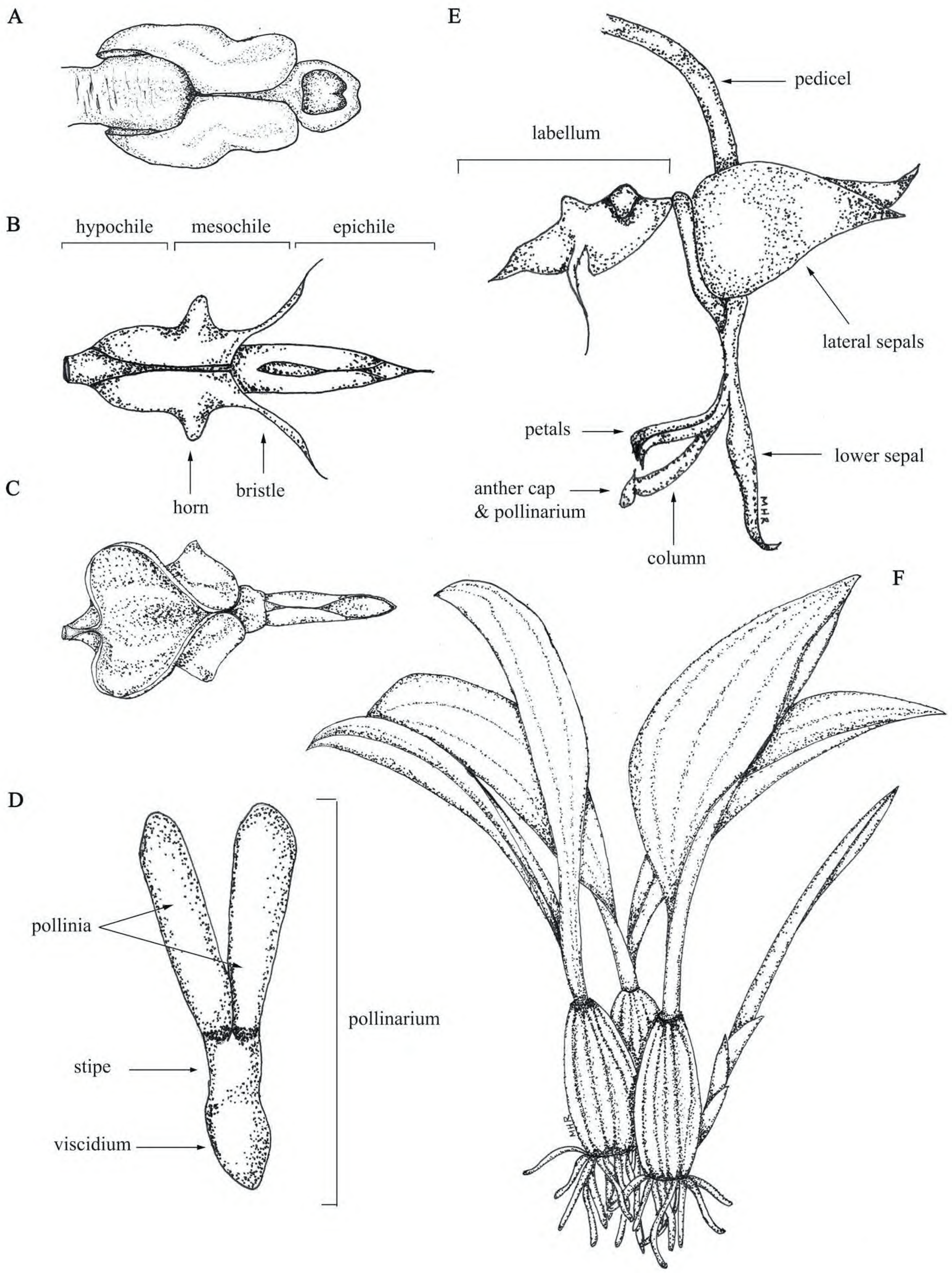


Figure 3. Vegetative and floral morphology of *Gongora* Ruiz & Pav. A–C. Adaxial side of labellum from three separate *Gongora* species. The labellum is divided structurally into three sections, the epichile, mesochile, and hypochile. The osmophores, or the collection of scent-producing cells, are located on the adaxial side of the hypochile. —A. *Gongora galeata* (Lindl.) Rchb. f. of *Gongora* subg. *Acropera*. The hypochile forms a broad and flexible attachment point to the rest of the flower, which allows the labellum to swing back during the hinge pollination mechanism. —B. Generalized *Gongora* species from section *Gongora* of *Gongora* subg. *Gongora*. —C. *Gongora tracyana* Rolfe in section *Truncata* of *Gongora* subg. *Gongora*. The hypochile flaps form a wide opening, which may prevent the pollinator (*Eulaeama bombiformis* Packard) from stealing floral volatiles. —D. A *Gongora* pollinarium consists of two pollen packets or pollinia (singular, pollinium), a stipe, and a sticky viscidium, which aids in the attachment of the pollinarium to the pollinator. —E. Generalized *Gongora* flower with floral structures labeled. —F. The vegetative form of a *Gongora* orchid. Several ovoid to conical pseudobulbs, which are characterized by pronounced vertical ridges, are connected by a short rhizome. Each pseudobulb bears two (rarely one) lanceolate-shaped leaves.

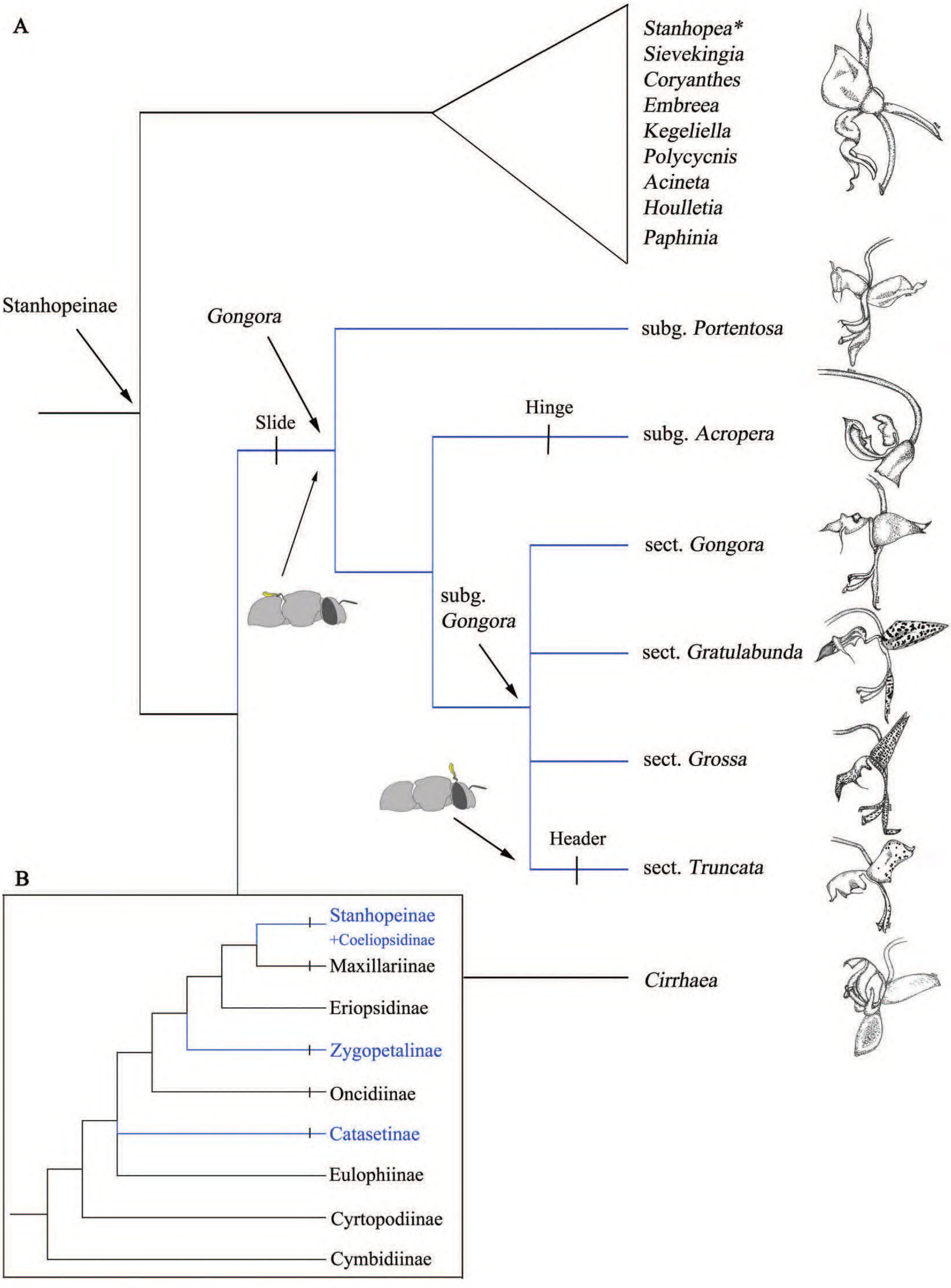


Figure 4. Phylogenetic relationships of *Gongora* Ruiz & Pav. and related genera. —A. Phylogenetic relationships of male euglossine bee–pollinated Stanhopeinae orchids, emphasizing the evolutionary relationships among subgenera of *Gongora* as supported by molecular data (adapted from Whitten et al., 2000). The subgenus *Gongora* is divided into four unresolved sections. The hypothesized origins of the three pollination mechanisms observed in the orchid genus *Gongora* are indicated, and the resulting placement of the pollinarium on the pollinator is illustrated. The line drawings to the right of each taxon terminal depict a representative specimen for that taxonomic group. The line drawing to the right of the collapsed clade is a representative specimen of the genus *Stanhopea* Frost ex Hook. (indicated with an asterisk). —B. Hypothesized phylogenetic relationships of

type specimen of *G. quinquenervis* Ruiz & Pav. The drawing of *G. quinquenervis* and the first description of the genus were published in 1794 in *Florae Peruvianae, et Chilensis Prodromus*; however, the type specimen of *G. quinquenervis* was not described until four years later in 1798 in *Systema Vegetabilium Florae Peruvianae et Chilensis* with the type locality indicated from Pozuzo, Peru (Ruiz & Pavon, 1794, 1798; Jenny, 1993, 1996). The remains of the type specimen are currently deposited in the Royal Botanical Garden of Madrid. There are no flowers associated with the type identification, making classification of the real *G. quinquenervis* difficult, if not impossible (Pupulin, 2012). Today the name *G. quinquenervis* is widely misapplied by many authors to describe otherwise unidentified specimens, and even pollinaria, collected from diverse localities well beyond Peru. Dressler (1968a) suggests that there are at least a dozen "good" species within the *G. quinquenervis* species complex.

Despite the taxonomic confusion, a classification system and genus-level revision are available. Rudolph Jenny (1993) recognized three subgenera: *Gongora* subg. *Gongora* (Pfitzer) Jenny, *Gongora* subg. *Acropera* (Pfitzer) Jenny, and *Gongora* subg. *Portentosa* Jenny (Fig. 4A). *Gongora* subg. *Acropera* was originally described by Lindley (1833), with the type *Acropera loddigessii* Lindl., as a separate genus based on the distinct floral morphology and the restricted geographic distribution in Central America (Lindley, 1833; Darwin, 1888; Jenny, 1993). However, modern phylogenetic analyses of the tribe Stanhopeinae using molecular data from nuclear ribosomal and plastid DNA supported the division of *Gongora* into three subgenera and revealed *Gongora* subg. *Portentosa* as sister to both *Gongora* subg. *Gongora* and *Gongora* subg. *Acropera* (Fig. 4A) (Whitten et al., 2000). It should be noted that these analyses emphasized the generic relationships of Stanhopeinae and included taxa sampled from across the tribe Cymbidieae and only nine species-level accessions of *Gongora*. Thus, these analyses were not intended to resolve species-level relationships. Based on the morphological characters of the flowers, Jenny (1993) divided *Gongora* subg. *Gongora* into four sections: *Gongora* sect. *Gongora*, *Gongora* sect. *Gratulabunda* Jenny, *Gongora* sect. *Grossa* Jenny, and *Gongora* sect. *Truncata* Jenny. However, the monophyly and evolutionary relationships among these sections remain unclear because commonly used gene sequences for phylogenetic inference have not exhibited enough sequence divergence at this level of resolution.

As currently arranged, *Gongora* sect. *Gongora* is the most species-rich group, with at least 30 recognized species, but is also the most difficult in which to delimit species boundaries, including those of the *G. quinquenervis* species complex (Whitten, 1985; Jenny, 1993). The ambiguity in species boundaries persists because multiple sympatric populations coexist with little to no morphological variation, but clearly experience reproductive isolation as inferred from the attraction of non-overlapping assemblages of male euglossine bee pollinators (Whitten, 1985; Hentrich, 2003; Ramírez, unpublished data). Pollinator identity within this *Gongora* species complex seems to be governed by differences in the floral scent chemistry (Whitten, 1985; Hentrich, 2003; Ramírez, unpublished data). Further, it remains unclear as to what extent pollinator identity and floral scent may co-vary within a single *Gongora* species across its geographic range.

Pollinator Specialization

Male euglossine bee–pollinated Orchidaceae exhibit intricate adaptations for pollinator attraction and cross-pollination, including strong floral scent, modified floral morphology, and specific mechanisms for the precise placement and attachment of pollinarium. The euglossine bee genera *Euglossa*, *Eufriesea*, and *Eulaema*, which encompass ca. 130, 66, and 30 species, respectively, are the most species-rich clades of euglossine bees and are therefore the main pollinators of most male euglossine bee–pollinated Orchidaceae (Ramírez et al., 2002, 2010; Nemésio & Rasmussen, 2011). *Gongora* exhibits adaptations for the attraction and pollination by euglossine bees, and because the flowers produce no additional rewards, these orchids are exclusively dependent on male euglossine bees for sexual reproduction.

Herein we review and examine the diversity of known pollinators of *Gongora*, for which we compiled an extensive dataset derived from both unpublished field observations and published literature of euglossine bee species that have been observed as (1) effective pollinators, (2) non-pollinating visitors, (3) pollinaria carriers, and/or (4) visitors with unknown pollination effect (Appendix 1). For consistency, we used the same classification—numbers (1) through (4) above—for denoting pollinators and visitors as used in

←
the tribe Cymbidieae (Orchidaceae). Black bars indicate lineages that contain male euglossine bee–pollinated orchid species, and blue lineages represent the three large and independent radiations of male euglossine bee–pollinated Orchidaceae (adapted from Ramírez et al., 2011 and Chase et al., 2015).

Ramírez et al. (2002). Because the same behavior by male bees that promotes the removal of the pollinarium leads to the subsequent deposition of the pollinia in the stigma (pollination), those bees that were collected carrying *Gongora* pollinaria in the field (3) can be considered actual pollinators, even if the pollination event was not observed; however, such observations do not permit species-level identification of *Gongora*, justifying the distinction between (1) and (3). Non-pollinating visitors (2) are assumed if there exists a size mismatch between that of the visiting male euglossine bee and *Gongora* species, whereas visitors with unknown pollination effect (4) refers to cases when visiting bees of the proper size do not pollinate the orchid during the time of observation. When available we provided *Gongora* species names, but due to the difficulty of correctly identifying morphologically similar species, the unavailability of voucher specimens for these orchids, and, in many cases, the lack of proper species names, we placed little significance on the species identity, particularly those described as *G. quinquenervis*. We also provided locality information for each observation when available in order to examine the extent of pollinator sharing and pollinator turnover across geographic ranges.

The results from these pollination observations (Appendix 1) are summarized in Figure 2A and B, in which we plotted the geographic location of each observed orchid–pollinator association. We also constructed a bee–orchid pollination network across several communities in Costa Rica and Panama (Fig. 2C). A total of 289 records describing 64 species of euglossine bees associated with *Gongora* were considered from a total of 50 localities distributed across nine countries in Central and South America. Species of *Euglossa* were among the dominant pollinators of *Gongora*, with 48 species of *Euglossa* listed as either true pollinators or pollinaria carriers. This number represents an impressive 37% of *Euglossa* species diversity. Furthermore, the total number of observations pertaining to *Euglossa* far exceeded those of other bee genera. In addition to species of *Euglossa*, five species of *Eufriesea* (of 66) were documented carrying pollinaria. In Costa Rica, Panama, and Brazil, four species of *Eulaema* (of 30) and one species of *Exaerete* (of eight) were found as either non-pollinating visitors or visitors with unknown pollination effect. However, those same species of *Eulaema* and *Exaerete* were observed carrying pollinaria in Peru, Ecuador, and Colombia. This observation suggests that in some instances bee visitor identity may remain constant, while pollination roles may vary across geography.

We constructed a pollination network based on the data gathered from multiple communities where *Gongora* orchids and their associated bee pollinators were recorded across five well-studied geographic regions in Costa Rica and Panama. We used the resulting network information to examine the turnover in orchid-pollinator associations across geography and to investigate the potential role that pollinator specialization may play in generating cryptic diversity within *Gongora* species complexes. This information is limited by the fact that the identification of *Gongora* species within these regions remains uncertain and the fact that many observations come from bees carrying *Gongora* pollinaria, in which case the species identity of *Gongora* cannot be determined from the pollinaria alone. Therefore, we grouped pollination observations by geographic region and had each orchid node in the pollination network represent a geographic region rather than a species identity of *Gongora*. Therefore, each orchid node of the network likely represents multiple potentially co-occurring species of *Gongora*. This approach affects only the resolution of the orchid nodes. In most cases the bee identifications were assumed to be accurate, and thus each bee node represents a single species of euglossine bee. Thus, our network analysis provides a rather conservative estimate of pollinator specialization.

The resulting mutualistic network indicates that there is both pollinator turnover (i.e., changes in pollinating bee species between orchid nodes) and pollinator sharing (i.e., consistency in pollinating species between orchid nodes) across geographic regions. The network also indicates that certain species of bee pollinators used by *Gongora* taxa are restricted to a single geographic region (i.e., a single pollinator is connected to only one orchid node), thus suggesting that a high level of pollinator specificity may exist across the landscape. Most pollination studies conducted with *Gongora* orchids at single locations have revealed that individual lineages exhibit highly specialized associations with few (ranging between one and four) species of euglossine bees (Dressler, 1968a; Whitten, 1985; Hentrich, 2003). Thus, the orchid nodes in our network that are connected to numerous bee pollinators likely represent multiple sympatric species of *Gongora*, where each reproductively isolated orchid species uses a subset of the pollinator pool (i.e., connected bee species nodes). This scenario is reinforced by the fact that we took a conservative approach in which we clustered sympatric orchid taxa into single nodes. In fact, our ongoing detailed studies on the pollination of *Gongora* orchids from the southern Pacific coast of Costa Rica indicate that multiple sympatric and

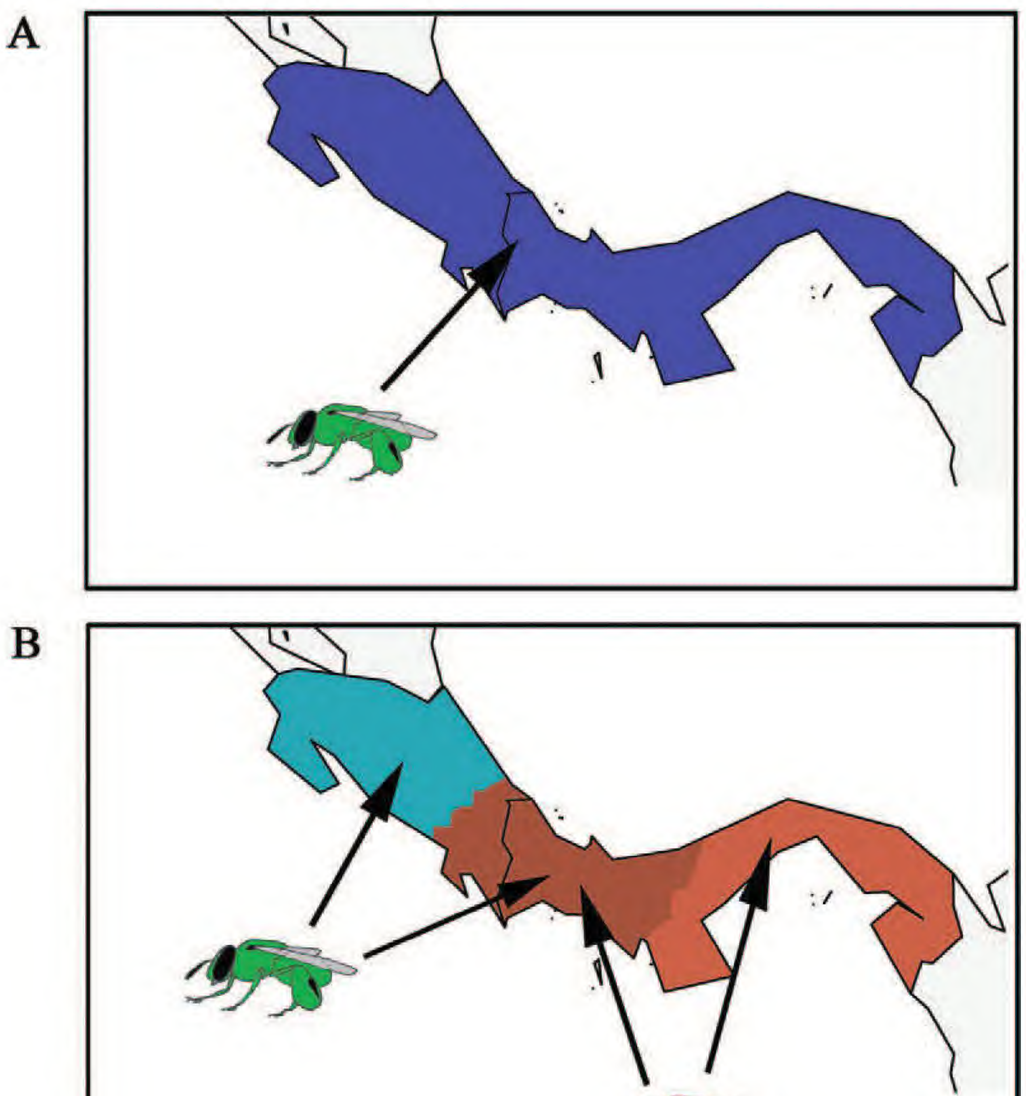


Figure 5. Alternative hypotheses governing gene flow across geography in terms of pollinator diversity and pollinator specialization. Filled colors represent geographically distinct populations of *Gongora* Ruiz & Pav. affiliated with specific pollinator assemblages. —A. Single *Gongora* population (blue) attracts the same pollinator species (hypothetical green bee) throughout its range. Gene flow is maintained across geography via long-distance pollen transfer. —B. Distant populations of *Gongora* (turquoise and orange) may be associated with distinct species of bee pollinators at opposite ends of the distribution (hypothetical green and red bees). In addition, populations of *Gongora* in the middle of the range (dark red) may attract and use both species of bee pollinators. The overlapping region in the middle of the range results in a porous barrier in which peripheral (turquoise and orange) populations experience gene flow with one another. However, this gene flow would be counteracted by the fact that peripheral populations may experience strong natural selection and thus local adaption to each pollinator species, leading to the fixation of distinct floral scent phenotypes. —C. Mosaic pattern of pollinator specialization in which two pollinator species are locally restricted (hypothetical green and red bees), while one pollinator species is widespread (hypothetical blue bee). The blue bee promotes gene flow between geographically separate *Gongora* populations (yellow and purple). Local adaptation to the restricted pollinators may persist.

cryptic *Gongora* species are reproductively isolated through the association with non-overlapping subsets of bee pollinators and that such pollinator specialization is mediated by the production of distinct floral scent profiles in each isolated orchid lineage.

The network architecture may be used in support of several non-mutually exclusive hypotheses to account for the role that pollinator diversity and pollinator specialization play across geography in generating and maintaining reproductive isolation among orchid lineages. These hypotheses are summarized in Figure 5. Euglossine bees are strong, long-distance fliers (Janzen, 1971; Ackerman et al., 1982), and mark-recapture experiments have shown that individual male bees may fly up to 72–95 km within a two-week period (Pokorny et al., 2014). Therefore, if pollinator species are shared among orchid populations, gene flow could persist even across long geographic distances (Fig. 5A). Thus, geographic isolation alone may not represent a strong reproductive isolating barrier when pollinator assemblages are shared. Alternatively, when the pollinator species used by *Gongora* taxa are restricted to a single geographic region, a reduction in gene flow between geographically isolated populations would be expected (Fig. 5B). This pattern could emerge from the process of local adaptation to specific pollinators, wherein a restricted orchid population has an altered scent phenotype that results in a shift to attract a local pollinator species. Although proper reciprocal transplant experiments remain to be conducted, some transplant experiments conducted in Panama revealed that single species of *Gongora* collected from one locality attracted separate and distinct pollinator assemblages at the collection locality and the transplant locality (Whitten, 1985). Furthermore, a mosaic pattern may emerge in which some of the pollinator species are locally restricted, while the remaining pollinators are widespread and shared between geographically separated populations (Fig. 5C). Under this scenario, gene flow among distant populations would be maintained, while local adaptation to restricted pollinators may persist. In summary, the observed patterns of pollinator specialization, high pollinator diversity, and variation in pollinator identity across geography together play an important role in the maintenance and formation of reproductive isolation and are likely responsible for the pervasive cryptic diversity observed in multiple species complexes of *Gongora*. A population genetic analysis on these populations is required to test these alternative hypotheses.

Pollination Mechanisms

Floral morphology plays a critical role in manipulating the behavior of insect pollinators while they visit

and forage on flowers. The family Orchidaceae has evolved a remarkably diverse array of floral traits that serve as adaptations for pollinator attraction and pollen transfer. In particular, highly modified structures like the labellum and the column have undergone exceptional modification that enables the precise placement and attachment of pollinaria on specific body parts of insect pollinators. The labellum, also referred to as the lip, is a modified petal that functions as a landing platform for insect pollinators (Dressler, 1981). In most orchids, as the flower bud develops, the pedicel twists, resulting in the relative orientation of the labellum below the column (resupinate) (Fig. 3E). The labellum of *Gongora*, however, is located above the column (non-resupinate), and in most species—with the exception of taxa belonging to *Gongora* subg. *Acropera*—the labellum is divided into three sections: the hypochile, mesochile, and epichile (Fig. 3B). Specialized scent-secreting cells (osmophores) are located on the adaxial surface of the hypochile and adjacent lateral sepals (Stern et al., 1986, 1987; Hentrich, 2004). At the beginning of anthesis, the osmophores are densely packed with starch granules, which are then rapidly metabolized over several days, fueling the production of scent volatiles (Vogel, 1963a, 1990). Both the production of floral scent by osmophores located on the labellum and the non-resupinate floral orientation of *Gongora* flowers ensure that male euglossine bee visitors hang upside down from the labellum with the head pointed toward the column while collecting volatile compounds. This positions them to successfully pollinate the orchid flowers.

The anther cap sits at the tip of the column and, once removed, it exposes the pollinarium (Dressler, 1981). The pollinarium is composed of two pollen packets—termed "pollinia"—that contain all of the flower's pollen grains and additional specialized associated structures (including the stipe and viscidium) that aid in the attachment of the pollinarium onto the bee's body (Fig. 3D) (Dressler, 1981; Singer et al., 2008). The viscidium is the sticky mass at the base of the pollinarium that glues the rest of the pollinarium onto the visiting pollinator. Once the pollinarium is removed by the pollinator, the stigma is exposed and is receptive to pollinia carried by a second pollinator from a donor flower. In this manner, *Gongora* flowers are dichogamous, i.e., they exhibit a reproductive strategy in which each flower contains both male and female reproductive structures, but the two phases are functionally separated in time. The time and distinction between the male and female phases are amplified by a period in which the pollinarium needs to dry before it can be successfully inserted into the receptive stigmatic opening (Whitten, 1985). This separation of sex by time, rather than by morphology, increases the opportunities for cross-fertilization (Dodson, 1962; Dressler, 1968b) and requires at least two visits by male euglossine bees to a *Gongora* orchid for successful pollination.

Male euglossine bees locate *Gongora* inflorescences from long distances and are aided entirely by the scent plume produced by the flowers (Dodson & Frymire, 1961; Dodson, 1967). Male euglossine bees first approach the *Gongora* inflorescence tentatively, moving toward and away from it while brushing the hind legs together in a stereotypical manner (Eltz, 2005). After circling the inflorescence for one to three minutes, the bee lands on a flower, although not always immediately in the correct position for pollination to occur. The male bee may brush floral volatiles from the sepals or from the sides of the labellum before maneuvering to the underside of the labellum (Hetherington-Rauth & Ramírez, pers. obs.). The bee uses the mid and hind legs to hold itself in place while secreting lipids from the labial glands to dissolve volatile compounds (Whitten et al., 1989; Eltz et al., 2007). It then brushes the surface of the flower using setae on the front tarsals and in doing so collects the volatile compounds (Fig. 6A) (Roubik & Hanson, 2004). After about 20 to 40 seconds of collection, the bee releases from the flower and hovers near the inflorescence while transferring the compounds into the hind leg pouches (Kimsey, 1984). The male bee then returns to the flower and repeats the process. A male bee may stay at a single *Gongora* inflorescence for up to 30 minutes, at which point it is hypothesized that the male bee becomes satiated on the volatiles and flies away.

Variation in the flower size and the floral morphology in *Gongora* has resulted in three distinct pollination mechanisms that differ in both the mode of attachment and location of attachment of the pollinarium onto the pollinator. We refer to these three mechanisms as the slide, the hinge, and the header mechanisms (Fig. 6). Both the slide and hinge mechanisms have been previously described in *Gongora* and result in the attachment of the pollinarium under the bee's mesoscutellum (Figs. 1, 6C, D). The header mechanism is described for the first time here and is exceptional in that the pollinarium is attached to the back of the head of the bee (Fig. 6F). Figure 4A depicts the hypothesized origin of each mechanism inferred from pollination observations and similarities in the floral morphology among subgenera and sections of *Gongora*.

The slide mechanism is well documented in the literature and has been described for at least two of the four sections of *Gongora* subg. *Gongora* including



Figure 6. Pollination mechanisms. A–C. Slide mechanism. —A. Male *Euglossa* cf. *cordata* uses the setae on the front tarsi (indicated by the arrow) to brush the surface of the labellum in order to gather floral volatiles. —B. Male *Eg. tridentata* Moure sliding down the column and removing the pollinarium. —C. Same *Eg. tridentata* from B after pollinarium removal. The pollinarium is attached under the mesoscutellum (indicated by the arrow). —D. Hinge mechanism. A male *Eg. viridissima* Friese pulls the labellum of *Gongora galeata* (Lindl.) Rchb. f. back and climbs atop the labellum to collect floral volatiles. As photographed, the labellum is fully extended. The dotted outline of the labellum indicates the initial position of the labellum. The double-headed arrow indicates the movement of the labellum as the bee pulls it back and then is released. In this photo the male bee has already removed the pollinarium, which is attached under the mesoscutellum (indicated by the arrow). E, F. Header mechanism. —E. A male *Eulaeama bombiformis* Packard grips the labellum of *G. tracyana* Rolfe in order to collect floral volatiles from its surface. The wide angle between the labellum and column accommodates the head of *El. bombiformis*. —F. The same *El. bombiformis* from E continues to collect floral volatiles after pollinarium removal. The pollinarium is attached to the back of the head (indicated by the arrow). Photos A–C by Tamara Pokorny taken in La Gamba, Costa Rica; photo D by Heiko Hentrich; photos E and F by Santiago Ramírez taken in Viterbo, Caldas, Colombia.

Gongora sect. *Gongora* and *Gongora* sect. *Grossa* (Allen, 1954; Dodson & Frymire, 1961; van der Pijl & Dodson, 1966; Dodson, 1967; van der Cingel, 2001). Although pollination has not yet been observed for *Gongora* subg. *Portentosa*, members of this group have a floral morphology similar to that of *Gongora* for which the slide mechanism has been described. Thus, it is likely that species in *Gongora* subg. *Portentosa* exhibit the slide mechanism. From this pattern we also infer the slide mechanism to be the ancestral pollination mechanism of the genus *Gongora* (Fig. 4A). Allen (1954: 123) first documented the slide mechanism in what he described as *G. maculata* Lindl. (in section *Gongora* of *Gongora* subg. *Gongora*) and described the bee as "a child on a toboggan" (123). In this mechanism, the bee maneuvers to the underside of the labellum where it brushes the surface (Fig. 6A). In the process of brushing, the bee slips and falls due to a combination of the labellum's slippery surface and the interference of the labellum's bristles. Upon falling, the male contacts the column and slides upon its complete curvature guided by the two petals, which act as guards on either side of the column (Fig. 6B). As the bee slides off the column, the sticky viscidium of the pollinarium catches the underside of the bee's mesoscutellum (Fig. 6C). The process of sliding down the column does not deter the bee from repeating the process. In fact, the male eagerly visits another flower and repeats the same behavior, although this time as the bee is sliding, the pollinia detach from the viscidium (leaving the hardened viscidium remains on the body) and is deposited into a receptive stigma.

The hinge mechanism has been described for the pollination of *Gongora galeata* (Lindl.) Rchb. f., by *Euglossa villosa* Moure. *Gongora galeata* flowers exhibit a pedicel with an extreme curvature and a highly flexible (or hinged) labellum, both of which aid in pollination (Rodriguez Flores et al., 1995). Members of *Gongora* subg. *Acropera* share both of these floral characteristics with *G. galeata*, and thus we infer that the hinge mechanism originated in the most recent common ancestor of *Gongora* subg. *Acropera* (Fig. 4A). The extreme curvature of the pedicel modifies the orientation of floral structures such that both the labellum and column are pointed upward (see line drawing of the representative specimen of *Gongora* subg. *Acropera* in Fig. 4A). The moveable (or hinged) labellum is no thicker than the sepals, resulting in a structure and attachment point that is highly flexible (Fig. 3A). Thus, without damaging the integrity of the structure, the labellum can be easily pulled back 90 degrees toward the pedicel. Upon release, the labellum returns to its resting position parallel to the column. In the hinge mechanism, the male bee uses the front and mid-legs to pull the upward-pointing labellum back toward the pedicel, thereby exposing the adaxial surface of the labellum, the source of floral volatiles (Fig. 6D). The male proceeds to climb on top of the adaxial surface and collect floral volatiles. At this point the bee is no longer holding the labellum against the pedicel. The pedicel, then, swings back into its resting position, placing the bee on its head with its thorax pushed up against the column. The orientation of the bee, column, and labellum at this point is highly reminiscent of gullet orchid flowers (in which the column and lip form a size-restricting chamber that the pollinator must enter in order to pollinate the flower) (Dressler, 1981). In this position, the viscidium catches and attaches to the underside of the mesoscutellum of the bee. By repeating this behavior at another flower, the bee deposits the pollinia into a receptive stigma.

The third mode, the header pollination mechanism, was observed by Ramírez (pers. obs.) for the first time in Viterbo (Caldas, Colombia) between *Gongora tracyana* Rolfe (section *Truncata* of *Gongora* subg. *Gongora*), the smallest species of *Gongora*, and the orchid bee *Eulaema bombiformis* Packard, the largest species of euglossine bee (Fig. 6E, F). If we only consider the slide mechanism, *El. bombiformis* would not be expected to successfully pollinate *G. tracyana* because it is too large to physically slide down the column and remove the pollinarium. Thus, it was a surprise when *El. bombiformis* was seen not only visiting *G. tracyana* but also removing the pollinaria and successfully pollinating the flowers as evidenced by the later development of seedpods. In this mechanism, *El. bombiformis* uses the hind and mid-legs to hang upside down beneath the labellum. Once in this position, *El. bombiformis* brushes the inside of the hypochile, which in *G. tracyana* is opened and rounded (Fig. 3C). The wide angle formed between the labellum and column accommodates the head of the bee, and the slight curvature of the column allows the viscidium to catch the back of the bee's head. After 15 to 30 seconds, *El. bombiformis* releases the labellum and hovers nearby the flower while transferring the volatile compounds to the hind tibiae. The bee does not slip or fall as is typical of the slide mechanism.

During the observation, the male *Eulaema bombiformis* occasionally gripped the lateral sepals with the mid- and hind legs while brushing at the sides of the labellum. While conducting this behavior, *El. bombiformis* was not in a position to pollinate *Gongora tracyana*. The wide flaps formed by the

hypochile of *G. tracyana* (Fig. 3C), however, likely hinder *El. bombiformis* from successfully collecting volatiles at the actual source of production and thus encourages *El. bombiformis* male bees to maneuver to the underside of the labellum in the correct position for pollination. In addition, during the pollination observation of *G. tracyana* in Colombia, we also observed males of *Euglossa* subg. *Euglossella* Moure visiting *Gongora* aff. *atropurpurea* Hook. (*Gongora* sect. *Grossa*), which was concurrently in flower. The *Euglossa* did not show any interest in *G. tracyana*, and *El. bombiformis* did not show any interest in *Gongora* aff. *atropurpurea*. Unfortunately, floral scent was not collected from either species of *Gongora* for chemical analysis. Nonetheless, each species of *Gongora* exhibited a perceivable odor that was distinct and that was not attractive to the other non-visiting species of bees. This clearly illustrates the extent to which floral scent can govern pollinator specificity even between *Gongora* species flowering side by side and suggests that floral scent may provide the greatest reproductive barrier between species.

Male *Eulaema* bees are not the typical pollinators of *Gongora* as their large size prevents them from maneuvering between the labellum and column (Appendix 1). Thus, species of *Gongora* pollinated by male *Eulaema* bees should exhibit changes in floral size, floral morphology, or both to accommodate the larger pollinator. This is exemplified by the header mechanism as described above. Such morphological changes accompanied by a change in pollinator size are highly reminiscent of what Dodson (1962) described as leap-frog speciation. Under the process of leap-frog speciation, the floral traits of one population experience strong selective pressures to adapt to a morphologically different (size) but possibly ethologically similar (scent preference) group of pollinators, resulting in two populations that are reproductively isolated (Dodson, 1962). Dodson suggested this as a hypothesis to describe a possible mode of speciation in *Stanhopea* Frost ex Hook. (also a member of the Stanhopeniae). He observed that sympatric populations of *Stanhopea* displayed differences in the width of the gap between the lip and column where male bees fall through after collecting floral volatiles and in doing so either remove or deposit the pollinarium (see line drawing accompanying *Stanhopea* genus in Fig. 4A). He also observed that the populations under study rarely hybridize, suggesting non-overlapping pollinator associations. He concluded the differences in morphology were driven by two morphologically different groups of pollinators, but which shared similar scent preferences. The smaller *Euglossa* bees selected for a narrow gap between the lip and column, whereas the larger *Eulaema* bees selected for a wide gap between the lip and column. In a similar sense, the ancestor of *G. tracyana* may have evolved a floral scent that attracted species of *Eulaema*. The large size of the visiting *Eulaema*, which may have on occasion accidentally acted as the pollinator, possibly drove the changes in floral morphology, resulting in what is today *G. tracyana*. Over evolutionary time, it is feasible that this leap to a new pollinator could result in the diversification of very small *Gongora* orchids pollinated by large *Eulaema* bees. In fact, several other species in *Gongora* sect. *Truncata* display similar floral morphology and floral size to that of *G. tracyana*, including *G. dresserli* Jenny, *G. longipes* Schltr., and *G. charontis* Rchb. f. It is likely that these taxa also exhibit the header mechanism for pollination. However, because not all species within *Gongora* sect. *Truncata* display floral traits similar to those of *G. tracyana*, we infer that the header mechanism originated within *Gongora* sect. *Truncata* (Fig. 4A).

Variation in the attachment site of pollinarium on the pollinator, as observed for *Gongora* (either attached under the mesoscutellum or attached to the back of the head), can act as a mechanical reproductive barrier, allowing for pollinator sharing between species with similar chemical compositions of floral scent (Hills et al., 1972; Ramírez et al., 2011). Among all genera of euglossine bee–pollinated Orchidaceae there are at least 13 different pollinarium attachment sites, and it is not uncommon to observe a single male euglossine bee carrying more than one pollinarium of different orchid genera attached to different locations on the body of the bee (Dodson & Frymire, 1961; Dressler, 1968b; Ackerman, 1983; Roubik & Hanson, 2004). Pollinarium placement, however, is often well conserved among orchid species of the same genus, suggesting that as a reproductive isolating mechanism, transitions between pollinarium attachment sites are less prevalent than changes in the chemical composition of floral scent (Ramírez et al., 2011). Nevertheless, the use of novel pollinarium attachment sites within the genus *Gongora* may provide opportunities for further diversification when coupled with changes in scent phenotype.

Concluding Remarks

Male euglossine bee pollination within Orchidaceae has a rich natural history that has and continues to capture the attention of evolutionary biologists, ecologists, and botanists. Until now, the

euglossine-pollinated genus *Gongora* has lacked an accessible and comprehensive review. This overview summarizes our current knowledge of the taxonomy, systematics, and pollination biology, including pollinator diversity and the different mechanical pollination modes. There are, however, several fundamental gaps to be filled in our knowledge regarding the evolutionary biology of *Gongora*. Firstly, a well-supported species-level phylogeny of the orchid genus will open the opportunity to test fundamental hypotheses about macroevolution, diversification rates, and floral trait evolution as driven by pollinator behavior. Secondly, more research is needed on the chemical ecology of floral scent. This research should investigate both the amount of interspecific and intraspecific variation of floral scent as well as the identity of floral volatiles that act as attractants and behavioral modifiers. Thirdly, the construction of pollination networks coupled with tools borrowed from population genetics should be implemented to understand the dynamic interaction between pollinator identity and geography and how this interaction influences gene flow between populations and contributes to the processes of local adaptation and speciation. *Gongora* orchids are remarkable in terms of their floral diversity and their intricate modes of pollination. Compared to most other genera of male euglossine bee–pollinated orchids, they are relatively common in some habitats, are easy to cultivate, and can be easily hybridized and grown from seed to flower within three to four years. The genus *Gongora* merits further attention and certainly provides a system in which to investigate multiple fundamental questions in evolutionary biology and ecology.

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Appendix 1. Pollination observations.

Pollination observations were gathered from existing publications (which cite personal field observations and/or museum and personal specimen collections of male euglossine bees with attached pollinaria) and unpublished personal field observations. The table is alphabetized first by country and then by locality within that country. Subsequent columns refer to the euglossine bee species name and bee behavior, as observed for *Gongora* Ruiz & Pav. taxa. Bee behavior is distinguished as four categories following the classification used in Ramírez et al. (2002): 1 = effective pollinator, 2 = non-pollinating visitor, 3 = pollinaria carrier, and 4 = visitor with unknown pollination effect. *Gongora* species names are given as listed in the accompanying reference. However, because of the difficulty of identifying *Gongora* species and the continual revision of the genus, caution should be taken in the interpretation of *Gongora* species identity, particularly for taxa identified as *G. quinquenervis* Ruiz & Pav. and for cases in which *Gongora* species identity is assigned based only on the pollinarium carried by euglossine pollinator without reference to an individual plant. Variety or chemotype refers to additional descriptions used by the referenced authors to further describe the (usually unnamed) *Gongora* species, especially when the species is not identified beyond *Gongora*. For a couple cases, the species variety or chemotype has since been assigned to its own taxonomic species; such cases are indicated with a footnote. We use *Gongora* sp. indet. to indicate that the *Gongora* species identity was unknown and/or not reported in the accompanying reference. We worked to include only the references for which independent and original observations were made, thus avoiding secondary citations. Within a reference, authors occasionally cited the unpublished observations made by others. When this occurred, we included the name of the observer identified in the reference in parentheses following the citation. Euglossine bee genera are abbreviated as follows: *Eufriesea* Cockerell (*Ef.*), *Euglossa* Latreille (*Eg.*), *Eulaema* Lepeletier (*El.*), and *Exaerete* Hoffmannsegg (*Ex.*).

With regard to observations referenced from Dressler's field notes:

Dressler kept meticulous field notes regarding euglossine pollinators. His field notes span the years of 1964–1978 and primarily focus on orchid and euglossine bee populations from Costa Rica and Panama. Dressler cultivated many of his orchids in Panama City, Panama, and/or Turrialba, Costa Rica (Dressler, 1968a). When the orchid flowered, he would take them into the field in a habitat that was as similar to their native habitat as possible. However, on some occasions Dressler took the orchid to a habitat where he had not naturally observed the orchid but which resembled the native habitat; he noted such cases. Many of his observations are referenced in his later publications. For example, his observations from Costa Rica and Panama that span from 1964 to 1966 were incorporated into his publication "Observations on orchids and euglossine bees in Panama and Costa Rica" published in 1968 in *Revista de Biologia Tropical* and into his publication "Some observations on *Gongora*" published in 1966 in *Orchid Digest*. Thus, references made to Dressler's publications may be redundant to references made to his field notes; nevertheless we decided to include both reference types. In the following table, we included both Dressler's field note number and the year of the observation.

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
Brazil	Belém	Pará	*Eg. amazonica*	3	*G.* sp. indet.		Dressler, 1982d
			Eg. chalybeata	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1193
			Eg. cordata	3	*G.* sp. indet.		Ducke, 1901
							Almir Rebello, pers. obs. in 1939, in Dressler's field notes, p. 112
							Dressler, pers. obs. in 1968, field note 1186
			Eg. mixta	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1183
			Eg. modestior	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1187
							Dressler, 1982c
			Eg. parvula	3	*G.* sp. indet.		Dressler, 1982b
			Eg. piliventris	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1177
			Eg. stilbonta	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1182
			Eg. viridis	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1205
Brazil	Conceição da Barra	Espírito Santo	*Ef. violacea*[1]	3	*G. bufonia*		Hoehne 1933
							Dressler, pers. obs. in 1968, field note 1272
			Eg. cordata	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1268
Brazil	Floresta de Tijuca	Rio de Janeiro	*Ef. violacea*	3	*G. bufonia*		Hoehne, 1933
							Dressler, pers. obs. in 1968, field note 1315
Brazil	Itacoatiara Road (Manaus)	Amazonas	*Eg.* aff. *crassipunctata*	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1165
Brazil	Linhares	Espírito Santo	*Eg. cordata*	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1286
Brazil	Refúgio Ecológico Charles Darwin, Atlantic Forest	Pernambuco	*Eg. cordata*	1	*G. quinquenervis*		Martini et al., 2003
			Eg. perpulchra	1	*G. quinquenervis*		Martini et al., 2003
			El. flavescens	2	*G. quinquenervis*		Martini et al., 2003
Brazil	Reserva Monte Pascoal	Bahia	*Eg. cordata*	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1256
Brazil	NA	Pará	*Eg. cordata*	1	*G. quinquenervis*		Ducke, 1901
Brazil	NA	NA	*Eg. cordata*	1	*G. quinquenervis*		Whitten, 1985
			Eg. ignita	1	*G. quinquenervis*		Whitten, 1985
Colombia	Bahía Solano	Chocó	*Eg. crassipunctata*	3	*G.* sp. indet.		Ramírez et al., 2011
			El. cingulata	3	*G.* sp. indet.		Ramírez et al., 2011
Colombia	Mocoa	Putumayo	*Eg. intersecta*	3	*G.* sp. indet.		Ramírez et al., 2011
Colombia	Viterbo	Caldas	*El. bombiformis*	1	*G. tracyana*		Ramírez, pers. obs. in 2014
			Eg. sp. indent. subg. *Euglossella*	1	*G.* aff. *atropurpurea*		Ramírez, pers. obs. in 2014
Colombia	Mocoa	Putumayo	*Eg. intersecta*	3	*G.* sp. indet.		Ramírez et al., 2011

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
Colombia	NA	NA	*Eg. ignita*	1	*G. quinquenervis*		Whitten, 1985
Costa Rica	Alajuela	Alajuela	*Eg. viridissima*	1	*G.* sp. indet.	"Guancaste"[2]	Dressler, 1968a
Costa Rica	Chitaría	Cartago	*Eg. cybelia*	1	*G.* sp. indet.	"Yellow Lip"[3]	Dressler, 1968a
			Eg. gorgonensis	1	*G.* sp. indet.	"Yellow Lip"[3]	Dressler, 1968a
			Eg. hansoni	3	*G.* sp. indet.	"Yellow Lip"[3]	Dressler, 1968a
			Eg. purpurea	1	*G. unicolor*		Dressler, pers. obs. in 1965, field note 220
			Eg. tridentata	3	*G.* sp. indet.		Dressler, pers. obs. in 1965, field note 225 Moure, 1970[4]
			El. cingulata	2	*G.* sp. indet.	"White Lip"	Dressler, pers. obs. in 1965, field note 222
			El. speciosa	4	*G.* sp. indet.	"Pale-Yellow Lip"	Dressler, pers. obs. in 1965, field note 227
Costa Rica	Golfito	Puntarenas	*Eg. dodsoni*	1	*G. quinquenervis*		Dodson, 1967
			Eg. dodsoni	2	*G.* sp. indet.	"Golfito"	Dressler, 1968a van der Pijl & Dodson, 1966
			Eg. flammea	1	*G.* sp. indet.	"Golfito"	Dressler, 1968a
			Eg. ignita	1	*G. quinquenervis*		Dodson, 1967
			Eg. ignita	4	*G.* sp. indet.		Dressler, pers. obs. in 1965, field note 258 van der Pijl & Dodson, 1966
Costa Rica	Guápiles	Limón	*Eg. gorgonensis*	1	*G.* aff. *quinquenervis*		Dodson & Hills, 1966
			Eg. gorgonensis	1	*G.* sp. indet.	"Yellow Lip"[3]	Dressler, 1968a (as observed by Dodson) van der Pijl & Dodson, 1966[5]
			Eg. purpurea	1	*G. unicolor*		Dodson & Hills, 1966 Dressler, 1966 Dressler, 1968a van der Pijl & Dodson, 1966[5]
Costa Rica	La Gamba	Puntarenas	*Eg. bursigera*	4	*G.* sp. indet.	chemotype S[6]	Ramírez, pers. obs. in 2014
			Eg. cf. *cordata*	4	*G.* sp. indet.	chemotype A[7]	Pokorny, pers. obs. in 2014
			Eg. cyanura	3	*G.* sp. indet.		Ramírez, pers. obs. in 2013
			Eg. erythrochlora	4	*G.* sp. indet.	chemotype M[8]	Pokorny, pers. obs. in 2013
			Eg. flammea	3	*G.* sp. indet.	chemotype S[6]	Ramírez, pers. obs. in 2013
			Eg. hansoni	4	*G.* sp. indet.	chemotype M[8]	Pokorny, pers. obs. in 2014
			Eg. imperialis	3	*G.* sp. indet.	chemotype A[7]	Pokorny, pers. obs. in 2014
			Eg. imperialis	3	*G.* sp. indet.		Ramírez, pers. obs. in 2013
			Eg. tridentata	4	*G.* sp. indet.	chemotype M[8]	Pokorny, pers. obs. in 2014 Ramírez, pers. obs. in 2013 Hetherington-Rauth & Ramírez, pers. obs. in 2014

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
			Eg. villosiventris	3	*G.* sp. indet.	chemotype A[7]	Ramírez, pers. obs. in 2013
			El. bombiformis	4	*G.* sp. indet.	chemotype A[7]	Pokorny, pers. obs. in 2014
			Ex. sp. indet.	4	*G.* sp. indet.	chemotype S[6]	Ramírez, pers. obs. in 2014
Costa Rica	La Selva Biological Station	Heredia	*Eg. azureoviridis*[9]	3	*G.* sp. indet.		Dressler, pers. obs. in 1974, field note 1570
			Eg. bursigera	3	*G.* sp. indet.		Dressler, pers. obs. in 1970, field note 1375
			Eg. dodsoni	3	*G.* sp. indet.		Dressler, pers. obs. in 1970, field notes 1370 &1376
			Eg. purpurea	3	*G.* sp. indet.		Dressler, pers. obs. in 1974, field note 1566
			Eg. purpurea	1	*G. unicolor*		Dodson, 1967[10] Dressler, 1966 Dressler, 1968a van der Pijl & Dodson, 1966[5]
Costa Rica	Las Cruces	Puntarenas	*Eg. gorgonensis*	3	*G. armeniaca*		Dressler, pers. obs. in 1968, field note 1094
			Eg. cybelia	4	*G.* sp. indet.	"Yellow Lip"[3]	Dressler, 1968a
			El. cingulata	2	*G.* sp. indet.	"Yellow Lip"[3]	Dressler, pers. obs. in 1964, field note 159
			El. speciosa	2	*G.* sp. indet.	"Yellow Lip"[3]	Dressler, pers. obs. in 1964, field note 158
Costa Rica	Palmar Sur	Puntarenas	*Eg. cordata*	1	*G. maculata*		Allen, 1954
			Eg. cordata	1	*G. quinquenervis*[11]		van der Pijl & Dodson, 1966 Dodson, 1967 Williams, 1982
Costa Rica	Puerto Viejo	Limón	*Eg. purpurea*	3	*G. unicolor*[12]		Dressler, 1968a
Costa Rica	Tilarán	Guancaste	*Eg. cordata*	1	*G. quinquenervis*		Dressler, pers. obs. in 1966, field note 485 Dodson, 1967 van der Pijl & Dodson, 1966
Costa Rica	Turrialba	Cartago	*Eg. gorgonensis*	1	*G.* sp. indet.	"Yellow Lip"[3]	Dodson, 1967[10] van der Pijl & Dodson, 1966[5]
			Eg. hansoni	1	*G.* sp. indet.	"Yellow Lip"[3]	Dodson, 1967[9] van der Pijl & Dodson, 1966[5]
			El. cingulata	2	*G.* sp. indet.	"Yellow Lip"[3]	Dodson, 1967[10] Dressler, 1968a van der Pijl & Dodson, 1966[5]
			El. nigrifacies	2	*G.* sp. indet.	"Yellow Lip"[3]	Dodson, 1967[10] Dressler, 1968a van der Pijl & Dodson, 1966[5]

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
			El. polychroma	2	*G.* sp. indet.	"Yellow Lip"[3]	Dressler, 1968a
							Dodson, 1967[10]
							van der Pijl & Dodson, 1966[5]
			El. speciosa	2	*G.* sp. indet.	"Yellow Lip"[3]	Dodson, 1967[10]
							Dressler, 1968a
							van der Pijl & Dodson, 1966[5]
Costa Rica	NA	NA	*Eg. azureovirdis*[9]	3	*G. quinquenervis*		Whitten, 1985
			Eg. bursigera	3	*G. quinquenervis*		Whitten, 1985
			Eg. dressleri	3	*G. quinquenervis*		Whitten, 1985
			Eg. hansoni	3	*G. quinquenervis*		Whitten, 1985
			Eg. hemichlora	1	*G. quinquenervis*		Whitten, 1985
			Eg. imperialis	3	*G. quinquenervis*		Whitten, 1985
			Eg. tridentata	1	*G. quinquenervis*		Whitten, 1985
			Eg. purpurea	1	*G. unicolor*		Dressler, 1968a
							Dressler, 1966
			Eg. variabilis	3	*G. quinquenervis*		Allen, 1950
			Eg. viridissima	1	*G. clavidora*		Jenny, 1993
			Eg. cf. *viridissima*	1	*G. armeniaca*		Dodson & Hills, 1966
Ecuador	Balsapamba	Bolívar	*Eg. viridissima*[13]	1	*G. maculata*[14]		Dodson & Frymire, 1961
Ecuador	Bilsa Biological Reserve	Esmeraldas	*Ef. chrysopyga*	3	*G.* sp. indet.		Ramírez et al., 2011
			Eg. tridentata	3	*G.* sp. indet.		Ramírez et al., 2011
			Ex. smaragdina	3	*G.* sp. indet.		Ramírez et al., 2011
Ecuador	Lita to Alto Tambo	Esmeraldas	*Eg. dresserli*	1	*G. hirtizii*		Dodson et al., 1989
Ecuador	Quevedo	Los Ríos	*Eg.* cf. *variabilis*	1	*G. quinquenervis*		Dodson, 1967[15]
							van der Pijl & Dodson, 1966[16]
			Eg. viridissima[13]	1	*G. malculata*[14]		Dodson & Frymire, 1961
Ecuador	Rio Palenque	Pichincha	*Eg. tridentata*	1	*G. quinquenervis*[17]		Dodson & Gentry, 1978
Ecuador	Tiputini River	Orellana	*Eg. fuscifrons*	3	*G.* sp. indet.		Ramírez et al., 2011
Ecuador	NA	NA	*Eg. gibbosa*	1	*G. grossa*		Dodson et al., 1989[18]
			Eg. hemichlora	1	*G. grossa*		Dressler, 1966
							Dodson, 1966
							Dodson, 1967
							Dodson et al., 1989
			Eg. ignita	1	*G. quinquenervis*		Dodson, 1967
							Whitten, 1985
			Eg. ioprosopa	3	*G. quinquenervis*		Whitten, 1985

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
			Eg. nigropilosa	1	*G. grossa*		Dodson, 1965 Dressler, 1966 Dodson, 1967 Dodson et al., 1989
			Eg. tridentata	1	*G. quinquenervis*		Whitten, 1985
			Eg. variabilis	3	*G. quinquenervis*		Allen, 1950 Whitten, 1985
Guyana	NA	NA	*Eg. cordata*	1	*G. quinquenervis*		Whitten, 1985
			Eg. ignita	1	*G. quinquenervis*		Whitten, 1985
Mexico	Coatepec	Veracruz	*Eg. villosa*	1	*G. galeata*		Rodriguez Flores et al., 1995
Mexico	Fortín de las Flores	Veracruz	*Eg. villosa*	1	*G. galeata*		Dressler, pers. obs. in 1966, field note 593
Mexico	Puerto Escondido Road	Oaxaca	*Eg. viridissima*	3	*G.* sp. indet.		Dressler, pers. obs. in 1966, field note 1479
Mexico	NA	NA	*Eg. viridissima*	1	*G. galeottiana*		Dressler, 1966
Panama	Barro Colorado Island	Panama	*Eg. allosticta*	3	*G. quinquenervis*		Ackerman, 1983
			Eg. azureoviridis[9]	3	*G. quinquenervis*		Ackerman, 1983
			Eg. bursigera	3	*G. quinquenervis*		Ackerman, 1983
			Eg. cordata	1	*G. maculata*		Ducke, 1901 Dressler, pers. obs. in 1965, field notes 241 and 248
			Eg. cordata	1	*G. quinquenervis*		Dressler, 1968a
			Eg. cyanaspis	4	*G. quinquenervis*		Ackerman, 1983 Dressler, 1968a
			Eg. cyanura	1	*G. tricolor*		Ackerman, 1983 Dressler 1968a[19]
			Eg. despecta	3	*G. quinquenervis*		Ackerman, 1983
			Eg. dodsoni	1	*G. quinquenervis*		Ackerman, 1983
			Eg. dressleri	3	*G. quinquenervis*		Ackerman, 1983
			Eg. hemichlora	1	*G. quinquenervis*		Ackerman, 1983 Dressler, 1968a
			Eg. hemichlora	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 927
			Eg. cf. *hemichlora*	3	*G. maculata*		Dressler, pers. obs. in 1965, field note 242
			Eg. heterosticta	3	*G. quinquenervis*		Ackerman, 1983
			Eg. imperialis	1	*G. maculata*		Dressler, pers. obs. in 1965, field note 246

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
			Eg. imperialis	3	*G. quinquenervis*		Ackerman, 1983
			Eg. mixta	3	*G. quinquenervis*		Ackerman, 1983
			Eg. townsendii	1	*G. maculata*		Dressler, pers. obs. in 1965, field note 245
			Eg. townsendii	1	*G. quinquenervis*		Ackerman, 1983 Dressler, 1968a
			Eg. tridentata	4	*G.* cf. *aromatica*[20]		Dressler, pers. obs. in 1965, field note 262 Moure, 1970[4]
			Eg. tridentata	1	*G. maculata*		Dressler, pers. obs. in 1965, field note 244 Moure, 1970[4]
			Eg. tridentata	1	*G. quinquenervis*		Ackerman, 1983 Dressler, 1968a
			Eg. tridentata	3	*G.* sp. indet.		Dressler, pers. obs. in 1964, field note 56 Moure, 1970[4]
			Eg. variabilis	3	*G. quinquenervis*		Ackerman, 1983
			Eg. viridis	4	*G. bufonia*	var. *latibasis*	Dressler, pers. obs. in 1964, field note 17
			Eg. viridis	4	*G. bufonia*	var. *tricolor*	Dressler, pers. obs. in 1964, field notes 9 and 12
			Eg. viridis	1	*G. tricolor*	"yellow"	Dressler, pers. obs. in 1965, field note 247
			Ex. smaragdina	2	*G. tricolor*		Dressler, 1968a[18]
Panama	Cerro Campana	Panama	*Eg. asarophora*	2	*G.* sp. indet.	"El Valle"[21]	Dressler, 1968a
			Eg. bursigera	3	*G.* sp. indet.	"La Selva"	Dressler, pers. obs. in 1970, field note 1427
			Eg. bursigera		*G.* sp. indet.		Dressler, pers. obs. in 1969, field note 1327
			Eg. deceptrix	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. deceptrix	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field notes 817, 943, 1013, and 1029 Dressler, pers. obs. in 1969, field note 1330 Dressler, pers. obs. in 1970, field note 1365
			Eg. gorgonensis	2	*G.* sp. indet.	"La Selva"	Dressler, pers. obs. in 1970, field note 1426
			Eg. gorgonensis	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field notes 823 and 1065 Dressler, pers. obs. in 1969, field note 1328
			Eg. heterosticta	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. heterosticta	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 1066
			Eg. mixta	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. nigrosignata	4	*G.* sp. indet.	"La Selva"	Dressler, pers. obs. in 1970, field note 1424
			Eg. tridentata	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. variabilis	3	*G. quinquenervis*		Roubik & Ackerman, 1987

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
			Eg. villosa	4	*G.* sp. indet.	"El Valle"[21]	Dressler, 1968a
			Eg. villosa	4	*G. gibba* × *G. quinquenervis*[22]		Dressler, pers. obs. in 1969, field note 1326
			Eg. villosa	4	*G. horichiana*		Dressler, pers. obs. in 1970, field note 1425
			El. bombiformis	2	*G. gibba* × *G. quinquenervis*[22]		Dressler, pers. obs. in 1969, field note 1334
Panama	Cerro Jefe	Panama	*Eg. heterosticta*	3	*G.* sp. indet.		Dressler, pers. obs. in 1974, field note 1521
Panama	Cerro Sapo	Darién	*Eg. tridentata*	3	*G.* sp. indet.		Dressler, pers. obs. in 1978, field note 1717
Panama	El Cope	Coclé	*Eg. deceptrix*	3	*G.* sp. indet.		Whitten, 1985
			Eg. tridentata	3	*G.* sp. indet.		Whitten, 1985
Panama	El Llano	Panama	*Eg. bursigera*	1	*G.* sp. indet.		Dressler, pers. obs. in 1974, field note 1506
			Eg. mixta	3	*G.* sp. indet.		Dressler, pers. obs. in 1974, field note 1510
			Eg. tridentata	3	*G.* sp. indet.		Dressler, pers. obs. in 1974, field note 1515
Panama	El Valle de Antón	Coclé	*Eg. asarophora*	2	*G.* sp. indet.	"El Valle"[21]	Dressler, 1968a
			Eg. cyanura	4	*G. tricolor*[23]	B[24]	Whitten, 1985[25]
			Eg. cyanura	1	*G. tricolor*		Dressler, 1968a[18]
			Eg. deceptrix	3	*G.* sp. indet.		Whitten, 1985
			Eg. deceptrix	4	*G. quinquenervis*[26]	A[27]	Whitten, 1985[25]
			Eg. deceptrix	4	*G. quinquenervis*	A[27]	Whitten, 1985
			Eg. despecta	3	*G. quinquenervis*	A[27]	Whitten, 1985
			Eg. dodsoni	4	*G. quinquenervis*[28]	B[29]	Whitten, 1985[25]
			Eg. gorgonensis	4	*G. quinquenervis*[26]	A[27]	Whitten, 1985[25]
			Eg. gorgonensis	4	*G. quinquenervis*	A[27]	Whitten, 1985
			Eg. gorgonensis	4	*G. quinquenervis*	B[29]	Whitten, 1985
			Eg. gorgonensis	3	*G.* sp. indet.		Whitten, 1985
			Eg. gorgonensis	1	*G.* sp. indet.	"El Valle"[21]	Dressler, 1968a
			Eg. hansoni	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 996
			Eg. nigrosignata	4	*G.* sp. indet.	"El Valle"[21]	Dressler, 1968a[19]
			Eg. tridentata	3	*G.* sp. indet.		Whitten, 1985
			Eg. variabilis	3	*G.* sp. indet.		Whitten, 1985
			Eg. variabilis	4	*G. quinquenervis*[26]	A[27]	Whitten, 1985[25]
			Eg. variabilis	4	*G. quinquenervis*	A[27]	Whitten, 1985
			Eg. villosa	4	*G.* sp. indet.	"El Valle"[21]	Dressler, 1968a
Panama	Gamboa	Panama	*Eg. dresserli*	1	*G. maculata*		Dressler, pers. obs. in 1965, field note 270
			Eg. viridis	4	*G. bufonia*	var. *tricolor*	Dressler, pers. obs. in 1964, field note 1
			Ex. smaragdina	4	*G. bufonia*	var. *latibasis*	Dressler, pers. obs. in 1964, field note 5

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
Panama	Las Cumbres	Panama	*Eg. cordata*	4	*G. maculata*		Dressler, pers. obs. in 1964, field note 19
			Eg. cordata	1	*G. quinquenervis*[30]		Dressler, 1968a
			Eg. cyanura	1	*G. tricolor*		Dressler, pers. obs. in 1968, field notes 901.5 and 903
			Eg. townsendii	1	*G. quinquenervis*[30]		Dressler, 1968a
			Eg. tridentata	1	*G.* sp. indet.	"Guancaste"[2]	Moure, 1970[4]
							Dressler, pers. obs. in 1965, field note 272
			Ex. smaragdina	4	*G. atropurpurea*[31]		Dressler, pers. obs. in 1965, field note 260
Panama	Madden Dam	Colón	*Eg. tridentata*	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 946
Panama	Pipeline Road	Colón	*Eg. allosticta*	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. bursigera	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. despecta	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. imperialis	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. mixta	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. tridentata	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. variabilis	3	*G. quinquenervis*		Roubik & Ackerman, 1987
Panama	Río Guanche	Colón	*Eg. tridentata*	3	*G.* sp. indet.		Dressler, pers. obs. in 1974, field note 1526
Panama	Río Iguanita	Colón	*Eg. asarophora*	3	*G.* sp. indet.		Whitten, 1985
			Eg. cyanaspis	3	*G.* sp. indet.		Whitten, 1985
			Eg. cyanura	4	*G. tricolor*[32]	B[24]	Whitten, 1985[25]
			Eg. deceptrix	3	*G.* sp. indet.		Whitten, 1985
			Eg. despecta	4	*G. quinquenervis*[33]	A[27]	Whitten, 1985[25]
			Eg. despecta	3	*G.* sp. indet.		Whitten, 1985
			Eg. dodsoni	4	*G. quinquenervis*[33]	A[27]	Whitten, 1985[25]
			Eg. hemichlora	4	*G. quinquenervis*[33]	A[27]	Whitten, 1985[25]
			Eg. imperialis	4	*G. quinquenervis*[33]	A[27]	Whitten, 1985[25]
			Eg. townsendii	4	*G. quinquenervis*[33]	A[27]	Whitten, 1985[25]
			Eg. tridentata	3	*G.* sp. indet.		Whitten, 1985
			Eg. tridentata	4	*G. quinquenervis*[33]	A[27]	Whitten, 1985[25]
			Eg. variabilis	3	*G.* sp. indet.		Whitten, 1985
			Eg. variabilis	4	*G. quinquenervis*[33]	A[27]	Whitten, 1985[25]
Panama	Santa Rita Lumber Road	Colón	*Eg. tridentata*	3	*G.* sp. indet.		Dressler, pers. obs. in 1970, field note 1388
Panama	Santa Rita Ridge	Colón	*Eg. allosticta*	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. asarophora	3	*G. gibba*		Roubik & Ackerman, 1987
			Eg. asarophora	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 853

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
			Eg. bursigera	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. deceptrix	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. dissimula	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. dressleri	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. heterosticta	3	*G.* sp. indet.		Dressler, pers. obs. in 1968, field note 998
			Eg. heterosticta	3	*G. quinquenervis*		Roubik & Ackerman, 1987
			Eg. tridentata	3	*G. quinquenervis*		Roubik & Ackerman, 1987
Panama	NA	NA	*Eg. azureoviridis*[9]	3	*G. quinquenervis*		Whitten, 1985
			Eg. bursigera	3	*G. quinquenervis*		Whitten, 1985
			Eg. cordata	1	*G. quinquenervis*		Dressler, 1966 Dodson, 1967[15] van der Pijl & Dodson, 1966[5]
			Eg. cyanura	1	*G. tricolor*		Dressler, 1966[18] Dressler, 1968a van der Pijl & Dodson, 1966[5] Dodson & Hills, 1966
			Eg. deceptrix	3	*G. quinquenervis*		Whitten, 1985
			Eg. despecta	3	*G. quinquenervis*		Ramírez et al., 2002 (Dressler, pers. obs.) Whitten, 1985
			Eg. hansoni	3	*G. quinquenervis*		Whitten, 1985
			Eg. heterosticta	3	*G. quinquenervis*		Whitten, 1985
			Eg. mixta	3	*G. quinquenervis*		Whitten, 1985
			Eg. orichalea[34]	1	*G. quinquenervis*		Dodson, 1967[10] van der Pijl & Dodson, 1966[5]
			Eg. townsendii	3	*G. quinquenervis*		Dodson, 1967[10] van der Pijl & Dodson, 1966[5] Whitten, 1985
			Eg. tridentata	1	*G. clavidora*		Jenny, 1993
			Eg. tridentata	1	*G. quinquenervis*		Ackerman, 1983 Roubik & Ackerman, 1987 Whitten, 1985
Peru	El Parque Nacional del Manu ("Panguana" Forest Reserve on Río Llullapichis)	Madre de Dios/ Cusco	*Eg.* cf. *parvula*	3	*G.* sp. indet.		Dressler, pers. obs. in 1975, field note 1607

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
Peru	Iquitos	Loreto	*Eg. augaspis*	1	*G. quinquenervis*		Dodson, 1967[15]
							Dodson & Hills, 1966
							van der Pijl & Dodson, 1966[16]
			Eg. cordata	1	*G. quinquenervis*		Dodson, 1967[15]
							Dressler, 1968a[35]
							van der Pijl & Dodson, 1966[16]
			Eg. decorata	1	*G. quinquenervis*		Dodson, 1967[15]
							Williams, 1982[36]
							van der Pijl & Dodson, 1966[16]
			Eg. ignita	4	*G. maculata*		Dodson, 1966[19]
			Eg. ignita	1	*G. quinquenervis*		Dodson, 1967[15]
							van der Pijl & Dodson, 1966[16]
			Eg. prasina	4	*G. maculata*		Dressler, 1982c
			El. cingulata	3	*G.* sp. indet.		Ramírez et al., 2011
Peru	Tarapoto	San Martín	*El. meriana*	3	*G.* sp. indet.		Ramírez et al., 2011
Peru	NA	NA	*Eg. augaspis*	1	*G. quinquenervis*		Whitten, 1985
			Eg. decorata	1	*G. quinquenervis*		Whitten, 1985
			Eg. ignita	1	*G. quinquenervis*		Whitten, 1985[37]
			Eg. modestior	3	*G. quinquenervis*		Whitten, 1985
Trinidad	NA	NA	*Eg. cordata*	3	*G. quinquenervis*		Dressler, 1968a
							Whitten, 1985
			Eg. ignita	1	*G. quinquenervis*		Whitten, 1985
NA	NA	NA	*Ef. anisochlora*	3	*G.* sp. indet.		Ramírez et al., 2002
			Ef. lucifera	3	*G.* sp. indet.		Ramírez et al., 2002
			Ef. rufocauda	3	*G.* sp. indet.		Kimsey, 1982
			Eg. asarophora	2	*G.* sp. indet.		Dodson, 1967[10]
			Eg. augaspis	1	*G. maculata*		Dodson, 1966
			Eg. bursigera	3	*G.* sp. indet.		Ramírez et al., 2002 (FLAS)
			Eg. cordata	1	*G. maculata*	var. *maculata*	Dodson, 1965
			Eg. cyanura	1	*G. fulva*		Dodson, 1965
			Eg. cyanura	1	*G. tricolor*		Dodson et al., 1969
			Eg. cyanura	1	*G. unicolor*		Dressler, 1966
							Jenny, 1993 (Chavez, pers. comm.)
			Eg. decorata	1	*G. quinquenervis*		Dodson, 1967
			Eg. decorata	1	*G. maculata*		Dodson, 1966
			Eg. dodsoni	1	*G. armeniaca*	var. *bicornuta*	Dodson, 1965

Appendix 1. Continued.

Country	Locality	Province/State/ Region	Bee species	Bee behavior	*Gongora* species	Variety or chemotype	References and observations
			Eg. dodsoni	4	*G. horichiana*		Dodson, 1966
			Eg. dodsoni	4	*G. maculata*		Ramírez et al., 2002 (Dressler, pers. obs.)
			Eg. despecta	3	*G. quinquenervis*		Ramírez et al., 2002 (Dressler, pers. obs.)
			Eg. gibbosa	3	*G. quinquenervis*		Dressler, 1982d
			Eg. gorgonensis	1	*G.* sp. indet.		Dodson, 1967[10]
			Eg. ignita	4	*G. maculata*		Dodson, 1966
			Eg. imperialis	1	*G. maculata*		Dodson, 1965
							Ramírez et al., 2002
			Eg. ioprosopa	4	*G.* sp. indet.		Dressler, 1982b
			Eg. oleolucens	3	*G. armeniaca*		Ramírez et al., 2002 (FLAS)
			Eg. prasina	3	*G. maculata*		Ramirez et al., 2002 (FLAS)
			Eg. purpurea	3	*G.* sp. indet.		Dressler, pers. obs. in 1974, field note 1566
			Eg. singularis	4	*G. maculata*		Ramírez et al., 2002 (FLAS)
			Eg. variabilis	3	*G.* sp. indet.		Ramírez et al., 2002 (RPSP)
			Eg. cf. *variabilis*	1	*G. grossa*		Dodson, 1965[38]
			Eg. violacea	1	*G. bufonia*		Hoehne, 1933
			Eg. viridis	3	*G. aromatica*		Ramírez et al., 2002 (FLAS)
			Eg. viridis	1	*G. maculata*	var. *tricolor*[39]	Dodson, 1965
			Eg. viridissima	1	*G. armeniaca*		Dodson, 1965
			Eg. viridissima	1	*G. grossa*		Dodson, 1962
			Eg. viridissima	1	*G. maculata*		Dodson, 1962
			Eg. cf. *viridissima*	4	*G. armeniaca*		Jenny, 1993 (Whitten, pers. comm.)
			Eg. cf. *viridissima*	1	*G. maculata*	var. *odoratissima*[40]	Dodson, 1965[38]
			El. cingulata	4	*G. atropurpurea*		Dodson, 1966
			El. cingulata	1	*G. scaphephorus*		Jenny, 1993[41]
			El. meriana	4	*G.* cf. *minax*		Dressler, 1979
			El. meriana	3	*G.* sp. indet.		Dressler, 1979

Abbreviations: FLAS, Florida Museum of Natural History Herbarium; RPSP, School of Philosophy, Sciences, and Letters, University of São Paulo, Brazil.

[1] The accompanying references apply the name *Euplusia violaceae*, which is no longer used and has since been named as *Eufriesea violaceae*.

[2] The *Gongora* that Dressler refers to as "Guancaste" and describes as being wine red in color and having a perceivable and strong clove oil scent is now the species description of *G. clavidora* Dressler (see Jenny, 1993).

[3] Whitten (pers. comm. in 2014) stated that the *Gongora* that Dressler referred to as "Yellow-Lip" is likely synonymous to the species name known presently as *G. odoratissma* Lemaire.

[4] Moure (1970) directly references the observations documented in Dressler's field notes.

[5] Van der Pijl and Dodson (1966) cite Dressler (1966) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Dressler (1966).

[6] This *Gongora* belongs to a population that is part of ongoing research. Individuals described as chemotype S produce a floral scent composition characterized by an unidentified monoterpene derivative and alpha farnesene as well as several other monoterpene compounds (unpublished data).

[7] This *Gongora* belongs to a population that is part of ongoing research. Individuals described as chemotype A produce a floral scent composition characterized by estragole and cis- and trans-methyl p-methoxycinnamate as well as other aromatic compounds (unpublished data).

[8] This *Gongora* belongs to a population that is part of ongoing research. Individuals described as chemotype M produce a floral scent composition characterized by terpinen-4-ol as well as other monoterpene compounds (unpublished data).

[9] The name *Eg. azureoviridis* Friese is no longer used and has since been named as *Eg. milenae* Bembé (see Bembe, 2005, 2007, and Nemésio & Rasmussen, 2011).

[10] Dodson (1967) cites Dressler (1966) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Dressler (1966).

[11] The accompanying references cite the original source as Allen (1954); however, Allen (1954) only mentions *Gongora malculata* with no mention of *G. quinquenervis*.

[12] This observation is based upon a pollinarium attached to the bee pollinator, and hence it is likely that the *Gongora* species name is inferred from the locality of the observation and the distribution of the named *Gongora* species.

[13] In addition to *Eg. viridissima*, Dodson and Frymire (1961) also state that they observed blue-purple species of *Euglossa* visiting the described *Gongora*.

[14] Dodson and Frymire (1961) apply the name *Gongora malculata*; however, the photographs included in the publication and locality of the observation suggest that the *Gongora* observed was more similar to *G. grossa*.

[15] Dodson (1967) cites Dodson (1962) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Dodson (1962).

[16] Van der Pijl and Dodson (1966) cites Dodson (1962) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Dodson (1962).

[17] Dodson and Gentry (1978) apply the name *Gongora quinquenervis* to the *Gongora* observed; however, they note that the *Gongora* observed may also be known as *G. malcualta* Lindl. and/or *G. superflua* Rchb. F., which they further suggest may prove to be synonymous names.

[18] Dodson et al. (1989) cites Williams (1982) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Williams (1982).

[19] The listed locality of the pollination observation is interpreted from the text, which describes either the distribution of the listed bee species and/or the distribution of the listed *Gongora* species.

[20] Dressler collected this *Gongora* cf. *aromatic* from Guancaste, Costa Rica (northernmost providence on the Pacific side of Costa Rica), and recorded pollination observation at Barro Colorado Island, Panama (central Panama).

[21] Whitten (pers. comm. in 2014) stated that the *Gongora* that Dressler referred to as "El Valle" is likely synonymous to the species name known presently as *G. powellii* Schlechter.

[22] Dressler purchased this *Gongora* hybrid from El Valle de Anton, Panama.

[23] Whitten (1985) collected this *Gongora tricolor* from Rio Iguanita, located in central Panama on the Atlantic side, and recorded pollination observation in El Valle de Anton, located on the Pacific side of western Panama.

[24] Whitten (1985) performed chemical analysis of the floral scent composition and revealed that the floral scent of what he referred to as *Gongora tricolor* B was characterized by the monoterpene, ipsdienol. Whitten (pers. comm. in 2014) states that *G. tricolor* B is synonymous to *G. fulva* Lindl.

[25] In order to investigate geographic variation in the species identity of euglossine bee pollinator and its role in generating and/or maintaining reproductive isolation among *Gongora* species, Whitten (1985) conducted several transplant experiments in Panama, in which he recorded euglossine bee visitors and/or pollinators of *Gongora* in the collection (native) locality and in the transplant locality. This observation was recorded at the transplant locality.

[26] Whitten (1985) collected this *Gongora quniquenervis* from El Boco del Torro, located on the Atlantic coast in western Panama, and recorded pollination observations in El Valle de Antón, located on the Pacific side of western Panama.

[27] Whitten (1985) performed chemical analysis of the floral scent composition and revealed that the floral scent of what he referred to as *Gongora quinquenervis* A was characterized by the aromatic compounds cis- and trans-methyl p-methoxycinnamate. Whitten (pers. comm. in 2014) states that *G. quinquenervis* A is synonymous to *G. powellii* Schltr.

[28] Whitten (1985) collected this *Gongora quinquenervis* from Golfito, located on the Pacific coast of southern Costa Rica, and recorded pollination observations in El Valle de Antón, located on the Pacific side of western Panama.

[29] Whitten (1985) performed chemical analysis of the floral scent composition and revealed that the floral scent of what he referred to as *Gongora quinquenervis* B was characterized by the monoterpene terpinen-4-ol. Whitten (pers. comm. in 2014) states that *G. quinquenervis* B is synonymous to *G. boracayanensis* Jenny, Dalström & W. E. Higgins.

[30] Dressler collected this *Gongora quinquenervis* from El Valle de Antón, located on the Pacific side of western Panama, and recorded pollination observations in Las Cumbres, located on the Pacific side of central Panama.

[31] Dressler collected this *Gongora atropurpera* Hook. from Peru and recorded pollination observations in Las Cumbres, Panama.

[32] Whitten (1985) collected this *Gongora tricolor* from Cerro Azul, located on the Pacific side of central Panama, and recorded pollination observations in Río Iguanita, located on the Atlantic side of central Panama.
[33] Whitten (1985) collected this *Gongora quinquenerivs* from El Valle de Antón, located on the Pacific side of western Panama, and recorded pollination observation in Río Iguanita, located on the Atlantic side of central Panama.
[34] The species name *Eg. orichalcea* is considered nomina nuda.
[35] Dressler (1968a) cites Dodson (1965) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Dodson (1965).
[36] Williams (1982) cites Dodson (1962) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Dodson (1962).
[37] Whitten (1985) cites Dodson (1962) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Dodson (1962).
[38] Dodson (1965) cites Dodson (1962) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Dodson (1962).
[39] *Gongora maculata* var. *tricolor* Lindl. is synonymous to *G. fulva* Lindl (see Jenny, 1993).
[40] *Gongora maculata* var. *odoratissima* Dodson is synonymous to *G. odoratissima* Lemaire (see Jenny, 1993).
[41] Jenny (1993) cites Dodson (1967) as the original source of the pollination observation; however, the pollination observation was not supported upon examination of Dodson (1967).

AVIAN DIVERSIFICATION PATTERNS ACROSS THE K-PG BOUNDARY: INFLUENCE OF CALIBRATIONS, DATASETS, AND MODEL MISSPECIFICATION[1]

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ABSTRACT

Birds represent the most diverse extant tetrapod clade, with ca. 10,000 extant species, and the timing of the crown avian radiation remains hotly debated. The fossil record supports a primarily Cenozoic radiation of crown birds, whereas molecular divergence dating analyses generally imply that this radiation was well underway during the Cretaceous. Furthermore, substantial differences have been noted between published divergence estimates. These have been variously attributed to clock model, calibration regime, and gene type. One underappreciated phenomenon is that disparity between fossil ages and molecular dates tends to be proportionally greater for shallower nodes in the avian Tree of Life. Here, we explore potential drivers of disparity in avian divergence dates through a set of analyses applying various calibration strategies and coding methods to a mitochondrial genome dataset and an 18-gene nuclear dataset, both sampled across 72 taxa. Our analyses support the occurrence of two deep divergences (i.e., the Palaeognathae/Neognathae split and the Galloanserae/Neoaves split) well within the Cretaceous, followed by a rapid radiation of Neoaves near the K-Pg boundary. However, 95% highest posterior density intervals for most basal divergences in Neoaves cross the boundary, and we emphasize that, barring unreasonably strict prior distributions, distinguishing between a rapid Early Paleocene radiation and a Late Cretaceous radiation may be beyond the resolving power of currently favored divergence dating methods. In contrast to recent observations for placental mammals, constraining all divergences within Neoaves to occur in the Cenozoic does not result in unreasonably high inferred substitution rates. Comparisons of nuclear DNA (nDNA) versus mitochondrial DNA (mtDNA) datasets and NT- versus RY-coded mitochondrial data reveal patterns of disparity that are consistent with substitution model misspecifications that result in tree compression/tree extension artifacts, which may explain some discordance between previous divergence estimates based on different sequence types. Comparisons of fully calibrated and nominally calibrated trees support a correlation between body mass and apparent dating error. Overall, our results are consistent with (but do not require) a Paleogene radiation for most major clades of crown birds.

Key words: Aves, birds, divergence dating, fossils, paleontology, phylogeny.

One of the enduring debates in avian evolution centers around the timing of the crown radiation (Feduccia, 1995; Cooper & Penny, 1997; Cracraft, 2001; Ericson et al., 2006, 2007; Brown et al., 2007, 2008; Brown & van Tuinen, 2011; Mayr, 2013, 2014a). A straightforward interpretation of the fossil record of birds suggests that only a handful of deep divergences (i.e., Neognathae–Palaeognathae, Galloanserae–Neoaves, Anseriformes–Galliformes, Anhimidae–Anatoidea, and Anseranatidae–Anatidae splits) occurred during the Cretaceous, followed by an explosive radiation after the Cretaceous–Paleogene (K-Pg) extinction. This hypothesis is supported by the virtual absence of convincing Cretaceous crown bird fossils; only the anseriform (waterfowl) species †*Vegavis iaai* has been widely accepted as a Cretaceous crown bird (Clarke et al., 2005, though see Mayr, 2013). Other Cretaceous fossil birds either represent archaic taxa that clearly fall outside of the crown radiation or controversial taxa such as †*Teviornis* and †*Polarornis* that are too incomplete to place securely within the context of avian phylogeny (Clarke, 2004; Clarke & Norell, 2004; Mayr, 2004; Longrich et al., 2011). In contrast, most molecular divergence dating analyses have supported an extensive radiation of crown clade birds during the Cretaceous (e.g., Cooper & Penny, 1997; Brown et al., 2008; Pacheco et al., 2011; Haddrath & Baker, 2012; Jetz et al., 2012, though see Ericson et al., 2006; Jarvis et al., 2014). Indeed, some results suggest the basal crown divergences within individual orders such as Charadriiformes (shorebirds;

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Baker et al., 2007) and Psittaciformes (parrots; Wright et al., 2008) had already occurred during the Cretaceous, which would imply that many major crown bird morphotypes and ecotypes evolved alongside non-avian dinosaurs, ecologically diverse stem birds, and pterosaurs and also that an exceptionally large number of avian lineages survived the K-Pg extinction. Confounding matters, many fossil calibrations within Aves have been criticized as inappropriate due to stem/crown conflation and other issues (e.g., Mayr, 2005a; Ksepka, 2009; Wijnker & Olson, 2009; Smith, 2015). Likewise, many studies have used Cretaceous-age minimum calibrations based on the tectonic rifting of New Zealand from Australia for splits within clades such as Passeriformes (songbirds) and Palaeognathae (ratites and tinamous) (e.g., Cooper et al., 2001; Ericson et al., 2002; Barker et al., 2004). This calibration strategy rejects the possibility of dispersal by volant basal songbirds and the presumed volant ancestors of kiwi and moa and so may drive overestimates of divergence ages if biogeographic branching patterns in these clades reflect overwater dispersal rather than more ancient vicariance events (Phillips et al., 2010; Mayr, 2013; Worthy et al., 2013). This is cause for concern because the majority of clades that make up New Zealand's modern avifauna can be clearly shown to have arrived by long-distance dispersal (Trewick & Gibb, 2010). Beyond the prevalence of overwater dispersal, the complex geological history of the region makes assigning a precise date for the geological separation of New Zealand and Australia difficult, and the final severance of a close connection via the Lord Howe Rise may not have taken place until the Paleocene (Cande & Stock, 2004).

In many regards, the debate over the early crown radiation of birds parallels the debate over the early radiation of placental mammals. All potential Cretaceous crown placental fossils are controversial, and the fossil record suggests most major divergences occurred rapidly in the aftermath of the K-Pg extinction (Simpson, 1945; Archibald & Deutschman, 2001; Wible et al., 2007; O'Leary et al., 2013). In contrast, many molecular dates imply an expansive Cretaceous radiation for placental mammals (Springer et al., 2003; Bininda-Emonds et al., 2007; Meredith et al., 2011, though see Kitazoe et al., 2007; dos Reis et al., 2012). As with birds, some fossil calibrations have been critiqued as inaccurately placed, potentially leading to dating overestimates (Benton et al., 2009; Phillips et al., 2009; Phillips, in press.).

True divergence dates cannot be directly observed but are likely to lie somewhere between the minimum ages indicated by fossils and the extreme end of the molecular age estimate spectrum. Although the fossil record of birds is more complete than commonly portrayed, the material from the critical Late Cretaceous interval is both scarce and poorly represented, e.g., many isolated and/or fragmentary specimens (Olson, 1985; Mayr, 2009; Longrich et al., 2011). Beyond this preservation issue, there are challenges to identifying fossils on the deepest branches of the avian crown. Many closely related extant clades of birds are starkly different in morphology; it would presumably be difficult to recognize a fossil from a species lying on the stem lineage leading to a group such as Afroaves (a clade uniting such disparate groups as rollers, kingfishers, woodpeckers, hornbill, hawks, and owls) because of the lack of recognizable skeletal synapomorphies. Thus, the possibility remains that some poorly understood Cretaceous fossils could belong on deep branches within the avian crown but remain unrecognized as such. Nonetheless, it seems increasingly unlikely that crown members of extant orders such as Psittaciformes or Passeriformes have been overlooked in Cretaceous deposits.

Given the unobtainable nature of true divergence ages, it is productive to explore patterns of disparity between molecular divergence estimates and fossil ages in order to seek potential biases. One underappreciated phenomenon is that disparity between fossil ages and molecular dates tends to be proportionally greater for shallower nodes in the avian Tree of Life (Ksepka et al., 2014). This is counterintuitive from the perspective of fossil preservation and identification. All other factors being equal, the quality of the fossil record is expected to be higher for more recent time horizons than for older time horizons because more fossil-bearing rocks are preserved from younger deposits (Raup, 1972). Thus, one would expect rock preservation bias on average to contribute to greater disparity at deep nodes, rather than shallow nodes as observed. Likewise, identification bias (failure to properly identify the oldest fossil of a clade) may also contribute to greater disparity at deep nodes: if paleontologists fail to recognize the oldest fossil of a given clade, that clade's stratigraphic range will be artificially shortened. As mentioned above, fossils on deeper branches may be hard to identify, because it is not possible to predict a priori when in the history of the lineage each diagnostic trait evolved. In contrast, fossils occupying younger branches (e.g., divergences within crown families) should be relatively straightforward to identify: all diagnostic characters shared by the common ancestor of extant representatives

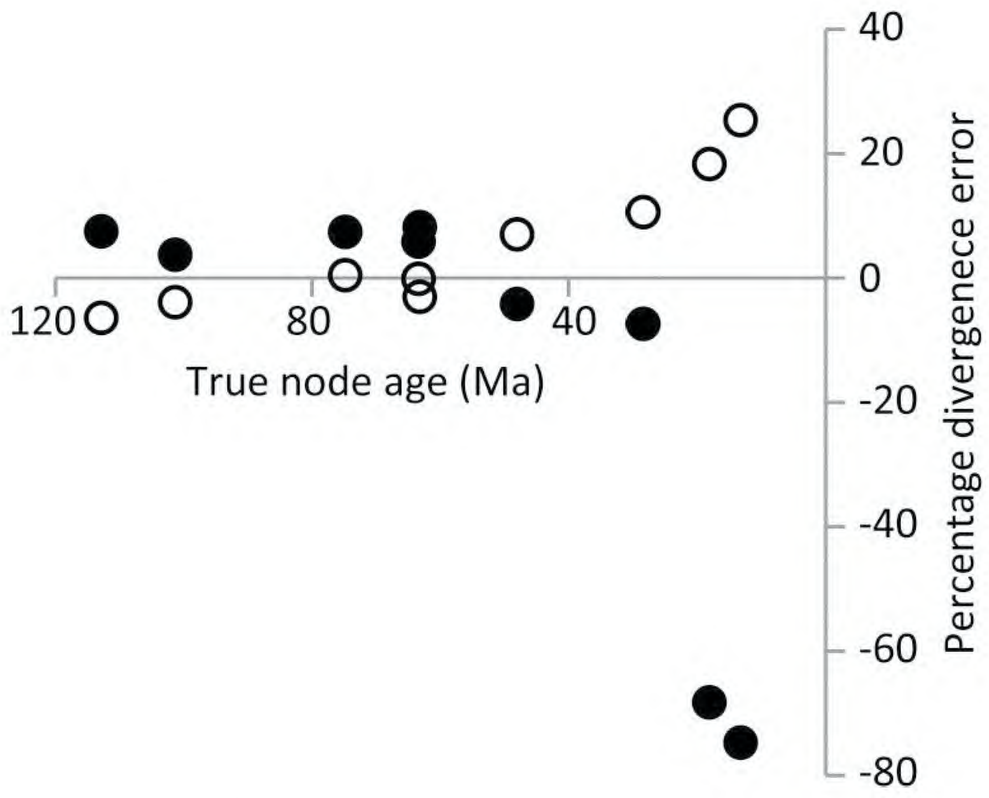


Figure 1. Divergence time errors (as a percentage of true node age) attributable to tree compression (open circles) and tree extension (closed circles) on a simulated 10-taxon tree pruned from the fully calibrated exon nDNA timetree. The horizontal axis represents divergence times simulated with the inferred alpha parameter for rate heterogeneity at 0.32. Tree compression was induced by undercorrecting for among-site rate heterogeneity (alpha = 2.0) and resulted in deep divergences being underestimated and shallow divergences overestimated. Tree extension was induced by overcorrecting for among-site rate heterogeneity (alpha = 0.1) and resulted in deep divergences being overestimated and shallow divergences underestimated. The horizontal scale indicates age as simulated in millions of years (Ma).

should be present in the oldest crown fossil. Although apomorphies of one of the two lineages branching from this common ancestor are technically required to prove crown status, paleontologists have tended to err in favor of assigning fossils to crown clades when the lack of such apomorphies leaves crown/stem status ambiguous. Conversely, loss of morphological features during fossilization can in some cases cause a specimen to erroneously appear in a more basal phylogenetic position, which could potentially lead to a crown taxon being misidentified as a stem taxon (Sansom et al., 2010, 2011; Sansom & Wills, 2013).

Another notable factor is that divergence dating analyses based on mitochondrial sequences have tended to yield older estimates than analyses based on nuclear sequences at least for ordinal level divergences (Ksepka et al., 2014). Clearly, both sets of dates cannot be correct, and some have considered mitochondrial dates problematic (van Tuinen & Hadly, 2004; Brown & van Tuinen, 2011). Thus, biases toward overestimation of mitochondrial DNA (mtDNA) dates and/or underestimation of nuclear DNA (nDNA) dates appear to exist in modeling substitution branch lengths, or in rate variation across the tree, at least at the ordinal level.

In sum, we contend that while biases in the fossil record are of major importance, they are unlikely drivers for many of the specific patterns of disparity observed across avian divergence dating studies. Therefore, exploration of potential drivers of disparity should also be sought among divergence dating analyses. One potential bias that has been widely discussed in relation to phylogeny is undercorrection or overcorrection for multiple nucleotide substitutions that occur at the same sites during the evolution of a lineage (e.g., Hendy & Penny, 1989; Siddall, 1998; Kolaczkowski & Thornton, 2004). Because this bias concerns underestimation or overestimation of branch lengths and becomes more pronounced more deeply in the tree, it will mislead divergence times. Branch-length underestimation leads to tree compression, with divergences that are shallower than calibrations being overestimated. Branch-length overestimation leads to tree extension, with divergences that are shallower than calibrations being underestimated. Both are illustrated in Figure 1 with analyses of simulated data and misspecification of substitution rate variation among sites.

Major sources of tree compression and extension suggested by Phillips (2009) included unaccounted-for rate heterogeneity among sites and substitution processes evolving across the tree (with models increasingly overcompensating for the amount of parallel changes between more divergent lineages). Although it is beyond the scope of this study to identify particular processes, a similar tree extension pattern might also be driven by alignment errors, which will manifest as increasing levels of branch-length overestimation as evolutionary depth increases and sequences become more difficult to align.

Several expectations are possible to derive here. One is that the higher saturation and heterogeneity of processes across substitution types in mtDNA versus nDNA (e.g., Wolfe et al., 1987; Hugall et al., 2007) provide greater potential for tree compression relative to nuclear sequences. However, that saturation and heterogeneity are greatly reduced by RY-coding (purines A+G → R, pyrmidines C+T → Y) and should reduce tree compression for mtDNA. If the potential for tree compression is sufficiently reduced, and if substitution processes evolve markedly across the tree, the balance may tip toward tree extension. In the case of birds (NT-coding predominates in most avian divergence dating analyses), such tree compression/tree extension artifacts may help explain at least some of the disparity observed between dates derived from nuclear and mitochondrial genes.

In this study, we examine patterns in avian molecular divergence estimation while controlling for phylogeny and aspects of the evolutionary models. Our aim is not to explicitly resolve avian divergence times, but rather to explore the influence of factors

such as gene type, the influence of single versus multiple calibrations relative to rate model alone, and relaxing the assumption of a molecular clock.

Materials and Methods

INSTITUTIONAL ABBREVIATIONS

The following abbreviations are used in this study: CM, Canterbury Museum, Christchurch, New Zealand; MACN, Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina; MGUH, Geological Museum of the University of Copenhagen, Denmark; MLP, Museo de La Plata, La Plata, Argentina; NHMUK, Natural History Museum, London, United Kingdom; NT, Tourment collection, Marseille, France; OCP.DEK/GE, Office Chérifien des Phosphates, Direction des Exploitations de Khouribga, Service Géologie, Khouribga, Morocco; PW, Landesamt für Denkmalpflege Rheinland-Pfalz, Mainz, Germany; QM, Queensland Museum, Brisbane, Queensland, Australia; SMF, Senckenberg Research Institute and Natural History Museum, Frankfurt am Main, Germany; SMNS, Staatliches Museum für Naturkunde, Stuttgart, Germany; USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C., U.S.A.

CALIBRATIONS

We utilized 15 node age priors (Appendix 1) that met recently proposed best practices for fossil calibrations (Parham et al., 2012). Several of these calibrations were discussed in greater detail by Ksepka and Clarke (2015) and Smith and Ksepka (2015). Each calibration was based on an individual fossil specimen that had been phylogenetically placed (assignable to the relevant node via either a published phylogenetic analysis or a set of apomorphies) and stratigraphically constrained so that the hard minimum age of the calibration corresponded to the minimum possible age of each calibrating specimen inclusive of geological dating error. We also specified maximum ages for each calibration based on global preservation patterns. Soft maxima are more subjective than hard minimum ages. In eight of our fossil calibrations, this greater uncertainty is accommodated by using an approximate lognormal distribution (i.e., a normal distribution truncated for the hard minimum at one standard deviation younger than the normal mean, with the soft maximum placed at the revised 2.5% upper bound). Such a distribution provides a wider region of high prior density, partly addressing one of the shortcomings of the lognormal distribution, while retaining the soft maximum, allowing 2.5% of the prior age distribution to surpass the maximum. In seven cases where the gap between our hard minimum and soft maximum ages is very wide and the fossil record cannot justifiably inform a prior distribution peak in the middle or either half of the distribution, we instead used uniform bounds. Uniform bounds are "hard" when used in the divergence analysis program, BEAST v.1.8.0 (Drummond & Rambaut, 2007). In addition to the fossil priors, a prior distribution for the root of the tree (basal split within Aves between Neognathae and Palaeognathae, node 1, Fig. 2) was specified with a hard minimum of 66.5 million years (Ma) and a soft maximum of 117.5 Ma, using a distribution that approximates the lognormal. This matches the root constraint used by Jetz et al. (2012).

MOLECULAR DATASET

Complete mitochondrial genome protein-coding DNA sequences (11,193 bp) and an 18-gene nuclear dataset comprising 7208 bp of protein-coding DNA (hereafter exon nDNA) and 14,691 bp of intron DNA (hereafter intron nDNA) and untranslated regions (UTRs) from Hackett et al. (2008) were aligned for a common set of 72 terminals. One additional gene (HMGN2) from Hackett et al. (2008) was excluded because it was missing for more than half of the taxa. All other genes were sampled at over 90% taxonomic completeness.

Taxa were selected to accommodate as many ordinal level clades as possible and to sample more densely within larger clades. In cases where loci were unavailable for a particular primary species, we included sequences from the closest available relative within either the genus or family (Appendix 2). This resulted in 21 cases where sequences from multiple species were combined into a composite terminal. Because different species provided the nuclear and mitochondrial sequences in many cases, a total of 142 species were sampled overall. Improved DNA matrix completeness can have substantial benefits in phylogenetic inference, while each member of the chimeric or composite taxon is theoretically equivalent for estimating phylogeny or divergence, so long as they form a clade relative to all other taxa in the matrix (Springer et al., 2004).

For the nDNA, we used the alignment of Hackett et al. (2008). For the mtDNA, automated alignment was performed in Muscle (Edgar, 2004a, 2004b) at EMBL (<http://www.ebi.ac.uk/Tools/msa/muscle/>) and then manually checked in Se-Al 2.0 (Rambaut, 1996). An important concern is the elevated mtDNA mutation rate, which amplifies both the impact of nucleotide composition biases and the difficulty of correcting for superimposed substitutions. As shown by Harrison et al. (2004), these problems are by far greatest at the largely synonymous third codon

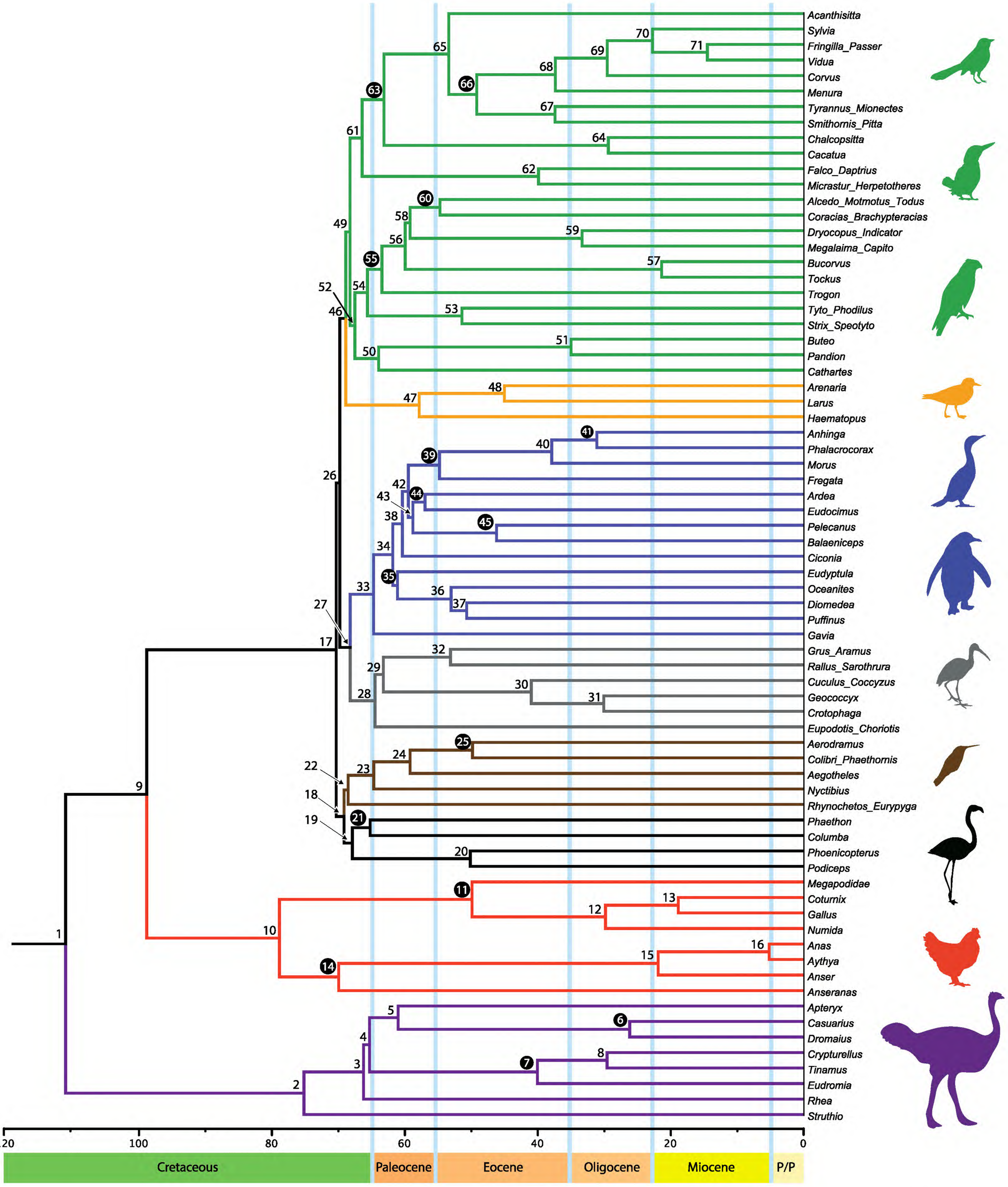


Figure 2. Timetree from divergence dating analyses using the preferred combined exon nDNA + RY-coded mtDNA dataset and applying all fossil calibrations. The horizontal scale below the diagram indicates age in millions of years, with dashed vertical lines indicating boundaries between geological time units. Node labels correspond to those in Tables 2–4 and Appendices 1 and 3. Filled circles indicate calibrated nodes.

positions, and so we excluded these from the mtDNA alignment. XML files containing the alignments, calibrations, and model settings are archived at Dryad (<doi:10.5061/dryad.3c3r3>).

ANALYSES

We estimated timescales for avian evolution using BEAST v.1.8.0 (Drummond & Rambaut, 2007) for both the nuclear exon and intron data and for the mitochondrial protein-coding data. The mtDNA was used as NT-coded (standard nucleotide sequence) or RY-coded to reduce phylogenetic signal erosion (see Phillips & Penny, 2003). Both the nuclear and mitochondrial protein-coding datasets were partitioned into codon positions. These partitioning schemes were preferred by the Bayesian information

Table 1. Partitioning schemes and substitution models.

Dataset	Partitions	Substitution model
NT mtDNA	codon positions	pos 1: GTR+I+G, pos 2: GTR+I+G
RY mtDNA	codon positions	pos 1: CF87+I+G, pos 2: CF87+I+G*
Exon nDNA	gene GC content × codon positions	low GC pos 1: TNR+I+G low GC pos 2: GTR+I+G low GC pos 3: GTR+G high GC pos 1: HKY+I+G high GC pos 2: GTR+I+G high GC pos 3: HKY+G
Intron nDNA	gene GC content	low GC: GTR+I+G high GC: GTR+I+G

Abbreviations: mtDNA, Mitochondrial DNA; nDNA, nuclear DNA.
* CF87+I+Γ4 (Cavender & Felsenstein, 1987) is the RY-coding equivalent to F81+I+Γ4.

criterion (in PartitionFinder 1.1.1, Lanfear et al., 2012) over concatenation or gene-wise partitioning. A combination of gene and codon partitioning was favored overall for the nuclear exons; however, with such high parameterization the analyses failed to converge. As a practical solution, we instead partitioned the nuclear genes into low and high GC content, which also tended toward low and high gamma distribution shape (alpha) parameters, respectively. Substitution model categories for each partition were assigned according to the most general of jModelTest (Posada, 2008) hierarchical likelihood ratio test (HLRT) and Akaike information criterion (AIC) recommendations (Table 1).

The same partitioning schemes were used for fully calibrated and nominally (root) calibrated relaxed clock analyses and for strict clock analyses. In the former cases, an uncorrelated relaxed clock model was used with rates among branches distributed according to a lognormal distribution, which can

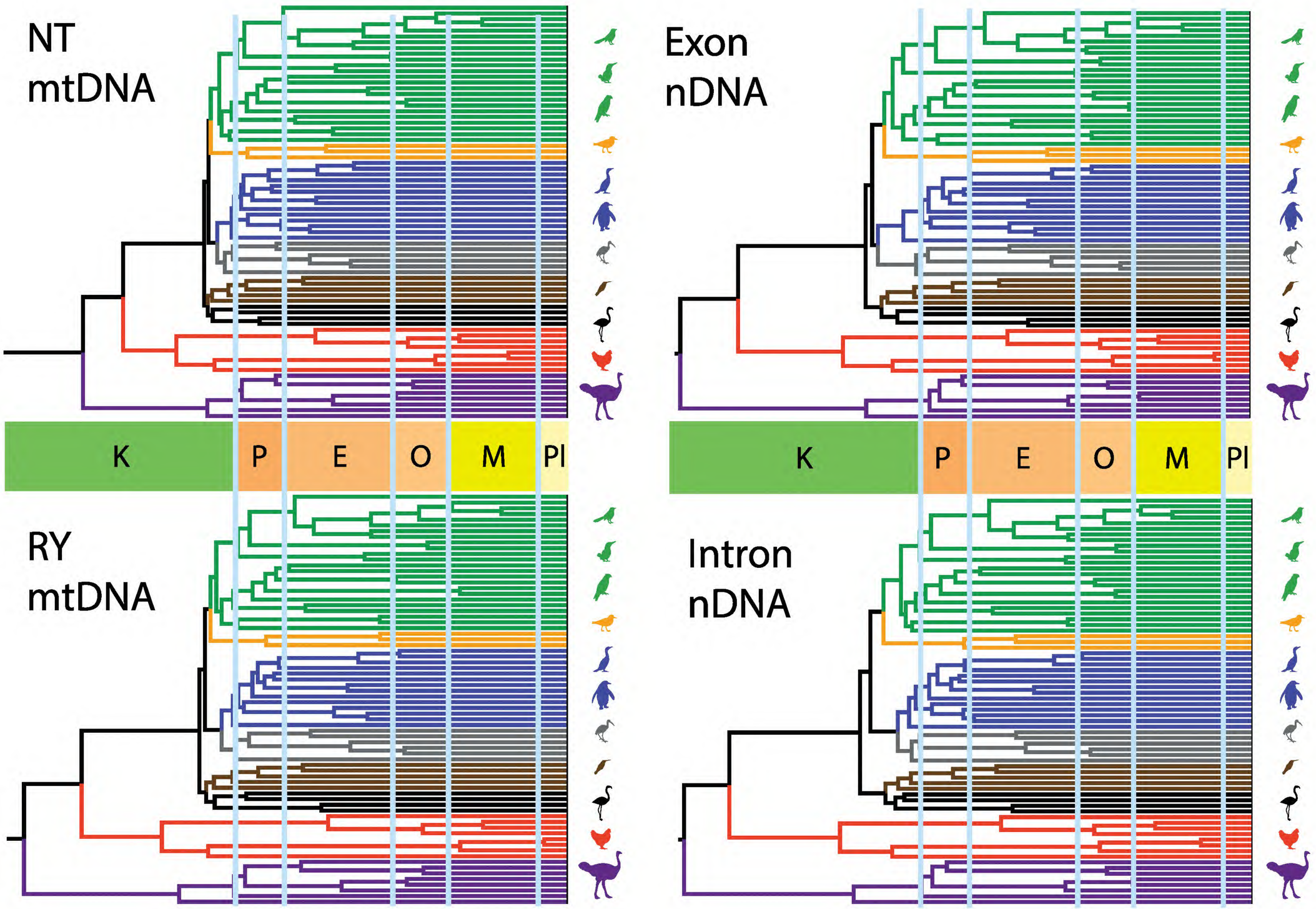


Figure 3. Timetrees from divergence dating analyses in BEAST with topology constrained and 15 fossil calibrations plus one root calibration applied comparing molecular datasets for NT- and RY-coding (A+G → R, C+T → Y) mtDNA as well as exon and intron/UTR nDNA.

Table 2. Median divergence age estimates (Ma) for all 71 nodes from fully calibrated divergence dating analyses. Combined analysis included coding nuclear DNA (nDNA) and RY-coded mitochondrial DNA (mtDNA). Node numbers correspond to those seen in Figure 2.

Node	Combined	Mitochondrial		Nuclear	
	Coding+RY	NT	RY	Exon	Intron
1. Aves (crown birds)	111.90	94.14	106.29	112.80	110.57
2. Palaeognathae (ratites and tinamous)	76.79	69.98	75.94	75.99	76.14
3. Notopalaeognathae (ratites excluding ostrich)	67.52	64.34	69.20	63.44	64.10
4. Tinamiformes/Novaeratitae	66.64	63.27	63.03	61.47	62.06
5. Novaeratitae (Apterygiformes [kiwi] + Casuariiformes)	62.39	56.65	52.06	56.94	59.13
6. Casuariiformes (cassowaries and emus)	26.36	29.96	28.10	28.07	28.46
7. Tinamiformes (tinamous)	40.02	45.53	44.13	35.96	35.59
8. *Crypturellus/Tinamus*	29.29	33.28	31.37	21.87	23.34
9. Neognathae (Galloanserae + Neoaves)	97.84	86.36	95.02	101.33	102.04
10. Galloanserae (Anseriformes + Galliformes)	78.47	75.99	79.31	80.59	80.39
11. Galliformes (landfowl)	49.41	48.96	46.78	48.03	49.13
12. Numididae (guineafowl)/Phasianidae (pheasants and allies)	29.91	30.97	28.24	29.63	30.73
13. *Gallus/Coturnix*	18.67	20.96	16.46	18.08	18.84
14. Anatoidea (magpie goose, ducks, and allies)	69.39	68.55	70.23	70.77	70.17
15. Anserinae (swans and geese)/other Anatidae	22.09	25.63	20.94	27.14	28.09
16. *Aythya/Anas*	5.16	11.44	4.52	7.01	9.45
17. Neoaves	70.41	70.61	71.96	76.11	76.44
18. Unnamed clade	69.24	69.89	70.65	72.98	71.76
19. Mirandornithes/other birds	68.40	68.94	68.97	69.79	69.90
20. Mirandornithes (Podicipediformes [grebes] + Phoenicopteriformes [flamingos])	50.05	59.81	48.08	43.91	45.66
21. Columbiformes (doves and pigeons)/Phaethontiformes (tropicbirds)	65.76	64.50	62.81	65.18	68.17
22. Eurypygiformes (kagu and subittern)/Strisores	68.65	69.04	69.12	71.18	69.34
23. Nyctibiidae (potoos)/Daedalornithes	64.81	65.90	65.55	66.45	66.96
24. Daedalornithes (Aegothelidae [owlet nightjars]/Apodiformes)	58.75	62.38	60.48	58.85	58.61
25. Apodiformes (swifts and hummingbirds)	49.71	51.31	51.72	49.68	50.04
26. Unnamed clade	69.88	69.76	70.89	74.80	74.47
27. Aequornithes (water birds)/other birds	68.32	68.05	67.62	73.65	69.24
28. Otidiformes (bustards)/other birds	64.64	66.57	65.07	66.12	66.56
29. Cuculiformes/Gruiformes	63.39	64.99	62.27	63.73	65.07
30. Cuculiformes (cuckoos)	41.75	50.05	42.45	39.30	44.36
31. *Geococcyx/Crotophaga*	30.94	42.10	31.83	25.63	31.88
32. Grues (cranes, trumpeters, and limpkin)/Rallidae (rails)	53.39	56.51	50.19	47.59	46.48
33. Aequornithes (waterbirds)	64.84	65.28	65.45	68.61	67.02
34. Austrodyptornithes/other Aequornithes	62.75	64.80	64.35	64.96	65.76
35. Austrodyptornithes (Sphenisciformes [penguins]+ Procellariiformes)	62.06	62.11	63.27	63.23	62.61
36. Procellariiformes (petrels and albatrosses)	53.68	55.63	45.64	47.63	56.41
37. *Puffinus/Diomedea*	51.36	50.06	39.11	42.78	54.35
38. *Ciconia*/other Aequornithes	61.34	63.42	61.59	63.62	63.14
39. Suliformes (frigatebirds, gannets, cormorants, and anhingas)	55.03	58.04	56.44	55.32	56.18
40. Sulidae (boobies and gannets)/Phalacrocoracoidea	38.32	48.57	40.88	39.16	38.40
41. Phalacrocoracoidea (cormorants and anhingas)	32.39	41.46	32.80	31.33	32.97
42. Suliformes/Pelecaniformes	59.67	62.93	60.53	60.79	61.52
43. Pelecaniformes (herons, ibises, pelicans, and allies)	58.99	61.21	59.20	59.45	58.92
44. Ardeidae (herons)/Threskiornithidae (ibises and spoonbills)	57.19	57.42	57.34	56.66	57.33
45. *Pelecanus/Balaeniceps*	46.52	48.79	42.98	43.91	43.09
46. Litoritelluraves (Charadriiformes + Telluraves)	68.98	69.31	69.78	72.42	71.92
47. Charadriiformes (shorebirds)	58.04	62.19	58.92	55.25	55.97
48. *Arenaria/Larus*	45.35	45.53	36.59	40.16	45.71
49. Telluraves (Afroaves + Australaves)	68.34	67.66	68.66	70.75	68.47
50. Cathartidae/Accipitres	64.48	65.66	64.51	61.81	66.31
51. *Buteo/Pandion*	35.42	45.53	36.70	31.35	36.12
52. Afroaves	67.63	67.18	67.62	68.66	67.50
53. Strigiformes (owls)	52.07	52.91	51.24	47.16	51.34
54. Strigiformes/Eucavitaves	66.14	65.22	65.34	66.42	65.64
55. Eucavitaves (Trogoniformes [trogons] + Picocoraciae)	63.60	62.28	61.57	63.35	63.79

Table 2. Continued.

Node	Combined	Mitochondrial		Nuclear	
	Coding+RY	NT	RY	Exon	Intron
56. Picocoraciae (Bucerotiformes + Picodynastornithes)	60.52	60.24	59.05	60.34	60.81
57. Bucerotiformes (hornbills)	22.33	31.28	20.99	23.93	27.17
58. Picodynastornithes (Coraciiformes + Piciformes)	59.02	58.66	56.94	58.66	58.02
59. Pici (woodpeckers and allies)	33.80	38.73	33.48	30.90	31.06
60. Alcedinidae (kingfishers)/Coracioidea (rollers and ground rollers)	54.97	54.57	54.41	54.27	53.16
61. Eufalconimorphae	66.94	64.21	64.44	68.66	64.79
62. Falconidae (falcons)	39.90	44.98	39.61	34.52	36.23
63. Psittacopasserae (Psittaciformes + Passeriformes)	63.70	62.77	62.14	62.19	62.60
64. Psittaciformes (parrots)	29.10	41.55	27.31	29.61	26.17
65. Passeriformes (songbirds)	53.66	55.23	53.33	52.51	51.45
66. Eupasseres (Tyranni + Passeri)	48.70	52.20	49.57	45.96	46.44
67. Tyranni (suboscine songbirds)	37.43	41.55	38.35	32.37	36.02
68. Passeri (oscine songbirds)	37.39	39.63	38.40	36.58	37.49
69. *Corvus*/other Passeri	29.27	31.47	29.00	28.40	28.29
70. *Sylvia*/*Vidua* + *Fringilla*	23.31	25.46	22.51	22.24	21.09
71. *Vidua*/*Fringilla*	14.35	16.50	12.86	13.30	15.08

provide greater flexibility than the exponential distribution option (Drummond et al., 2006). Birth–death tree priors were utilized for strict and relaxed clock analyses. The phylogeny of Hackett et al. (2008) was fixed for all analyses to ensure the comparability of divergence estimates from the alternative datasets and treatments.

Between two and 24 independent runs of 15 million Markov chain Monte Carlo (MCMC) generations were required for each analysis to provide estimated sample size values greater than 100 (as estimated in Tracer v1.5; Rambaut & Drummond, 2007) for all node height, prior, posterior, $-\ln L$, tree, and substitution parameters. Chains were sampled every 4000 generations after burn-ins of variable length, depending on convergence. We also conducted an analysis using a combined nuclear exon and RY-coded mtDNA dataset. We favor these datasets because RY-coding reduces compositional biases and phylogenetic signal erosion associated with the mtDNA (see above), and the nuclear intron data are expected to be the most susceptible to misalignment (see Morgan-Richards et al., 2008).

APPARENT DATING ERROR

In order to quantify the degree to which the fossil calibrations influence dates beyond providing a timescale, we compared the fully calibrated tree from each analysis to a scaled nominally calibrated tree based on the same underlying dataset. Each nominally calibrated tree used the same root calibration as the fully calibrated analyses, but excluded the other 15 fossil calibrations. We then scaled each nominally calibrated tree to minimize the sum of node height differences from the equivalent fully calibrated tree (e.g., multiplied all node dates from the nominally calibrated timetree by the single factor that results in the smallest overall difference in ages across all nodes between that nominally calibrated timetree and its fully calibrated equivalent). This scaling serves to counteract the possibility of any one divergence (such as the root) erroneously shifting the rest of the dates in one direction. Apparent dating error is equal to the difference in node ages between a fully calibrated tree and a presumably less accurate nominally calibrated tree (Phillips, in press). This provides a measure of how much a set of multiple calibrations informs the model of molecular rate variation and (presumably) corrects the relative dates given by relaxing the clock alone. We use the term "apparent dating error" for this measure because the true dates are, of course, unknown.

BODY MASS DATA

We collected average body mass data from the *CRC Handbook of Avian Body Masses* (Dunning, 2007), averaging data from multiple species in the case of composite taxa (Appendix 3). To test for an association between substitution rate misspecification and body mass, we compared log body mass to apparent dating error for nodes represented by pairs of tip taxa. Correlations between percent apparent dating error and log body mass for these nodes were evaluated using Pearson's product-moment correlation coefficient via the cor.test() function in R.

Table 3. Median divergence age estimates (Ma) for all 71 nodes from nominally calibrated divergence dating analyses. Node numbers correspond to those seen in Figure 2.

	Nominally Calibrated				Scaled Nominally Calibrated			
	Mitochondrial		Nuclear		Mitochondrial		Nuclear	
Node	NT	RY	Exon	Intron	NT	RY	Exon	Intron
1. Aves	81.31	81.08	81.20	80.86	94.89	122.27	139.34	121.21
2. Palaeognathae	61.24	53.45	48.54	50.86	71.47	80.60	83.29	76.24
3. Notopalaeognathae	56.46	44.74	39.94	41.75	65.89	67.47	68.54	62.58
4. Tinamiformes/Novaeratitae	55.59	42.93	38.81	40.27	64.87	64.74	66.60	60.36
5. Novaeratitae	49.12	31.24	35.82	38.10	57.32	47.11	61.47	57.11
6. Casuariiformes	18.50	4.88	7.54	10.76	21.59	7.36	12.94	16.13
7. Tinamiformes	41.01	30.06	21.88	23.69	47.86	45.33	37.55	35.51
8. *Crypturellus/Tinamus*	30.61	21.41	13.35	15.77	35.72	32.29	22.91	23.64
9. Neognathae	71.90	67.45	66.53	71.31	83.91	101.71	114.17	106.89
10. Galloanserae	59.20	51.79	49.18	53.10	69.09	78.10	84.39	79.60
11. Galliformes	48.22	39.69	37.43	42.12	56.27	59.85	64.23	63.14
12. Numididae/Phasianidae	30.08	21.86	21.27	25.30	35.10	32.96	36.50	37.92
13. *Gallus/Coturnix*	20.58	12.30	12.59	14.32	24.02	18.55	21.60	21.47
14. Anatoidea	47.17	36.98	37.66	39.51	55.05	55.77	64.62	59.23
15. Anserinae/other Anatidae	20.75	12.17	14.79	16.50	24.22	18.35	25.38	24.73
16. *Aythya/Anas*	9.11	2.94	3.91	6.32	10.63	4.43	6.71	9.47
17. Neoaves	61.11	49.03	44.35	51.70	71.32	73.94	76.10	77.50
18. Unnamed clade	60.53	47.35	42.07	49.03	70.64	71.40	72.19	73.50
19. Mirandornithes/other birds	59.64	46.02	40.60	48.00	69.60	69.40	69.67	71.95
20. Mirandornithes	50.24	29.97	26.61	31.37	58.63	45.19	45.66	47.02
21. Columbiformes/Phaethontiformes	55.61	39.82	38.41	47.15	64.90	60.05	65.91	70.68
22. Eurypygiformes/Strisores	59.60	45.82	41.14	47.15	69.55	69.10	70.60	70.68
23. Nyctibiidae/Daedalornithes	56.21	41.56	36.24	45.22	65.60	62.67	62.19	67.78
24. Daedalornithes	52.70	36.08	29.24	37.74	61.50	54.41	50.18	56.57
25. Apodiformes	40.39	26.22	20.23	30.00	47.14	39.54	34.71	44.97
26. Unnamed clade	60.35	48.23	43.59	50.13	70.43	72.73	74.80	75.14
27. Aequornithes/other birds	58.30	40.32	42.50	42.19	68.04	60.80	72.93	63.24
28. Otidiformes/other birds	56.77	38.42	38.16	40.38	66.25	57.94	65.48	60.53
29. Cuculiformes/Gruiformes	55.24	36.54	36.90	39.39	64.47	55.10	63.32	59.05
30. Cuculiformes	43.51	24.96	22.57	27.86	50.78	37.64	38.73	41.76
31. *Geococcyx/Crotophaga*	35.67	18.44	15.11	20.49	41.63	27.81	25.93	30.71
32. Grues/Rallidae	47.23	28.94	27.81	27.62	55.12	43.64	47.72	41.40
33. Aequornithes	52.72	34.96	32.82	39.85	61.52	52.72	56.32	59.74
34. Austrodyptornithes/other Aequornithes	52.16	33.47	27.52	38.72	60.87	50.47	47.22	58.04
35. Austrodyptornithes	47.55	32.12	25.90	35.11	55.49	48.44	44.44	52.63
36. Procellariiformes	40.98	23.96	19.94	30.46	47.82	36.13	34.22	45.66
37. *Puffinus/Diomedea*	36.49	20.59	17.62	29.10	42.58	31.05	30.24	43.62
38. *Ciconia*/other Aequornithes	50.96	30.42	26.58	36.69	59.47	45.87	45.61	55.00
39. Suliformes	46.37	26.18	20.31	31.18	54.11	39.48	34.85	46.74
40. Sulidae/Phalacrocoracoidea	38.61	18.40	13.80	21.40	45.06	27.75	23.68	32.08
41. Phalacrocoracoidea	32.93	14.10	10.48	18.08	38.43	21.26	17.98	27.10
42. Suliformes/Pelecaniformes	50.52	29.48	24.36	35.49	58.96	44.46	41.80	53.20
43. Pelecaniformes	48.90	27.99	23.42	33.43	57.07	42.21	40.19	50.11
44. Ardeidae/Threskiornithidae	44.27	25.67	20.75	32.22	51.66	38.71	35.61	48.30
45. *Pelecanus/Balaeniceps*	40.13	19.03	16.72	23.43	46.83	28.70	28.69	35.12
46. Litoritelluraves	60.01	47.69	42.59	48.49	70.03	71.92	73.08	72.69
47. Charadriiformes	53.15	36.74	31.97	36.84	62.03	55.40	54.86	55.22
48. *Arenaria/Larus*	39.18	22.09	23.99	30.50	45.72	33.31	41.17	45.72
49. Telluraves	58.84	47.15	41.90	46.26	68.67	71.10	71.90	69.34
50. Cathartidae/Accipitres	56.83	43.63	35.39	44.64	66.32	65.79	60.73	66.92
51. *Buteo/Pandion*	38.46	23.56	18.10	21.63	44.88	35.53	31.06	32.42
52. Afroaves	58.36	46.32	40.52	45.50	68.11	69.85	69.53	68.20
53. Strigiformes	44.05	33.28	26.41	32.66	51.41	50.19	45.32	48.96

Table 3. Continued.

Node	Nominally Calibrated				Scaled Nominally Calibrated			
	Mitochondrial		Nuclear		Mitochondrial		Nuclear	
	NT	RY	Exon	Intron	NT	RY	Exon	Intron
54. Strigiformes/Eucavitaves	56.38	44.50	39.24	43.96	65.80	67.11	67.34	65.90
55. Eucavitaves	53.64	41.30	37.67	42.40	62.60	62.28	64.64	63.56
56. Picocoraciae	51.41	38.11	34.66	39.27	60.00	57.47	59.48	58.87
57. Bucerotiformes	26.44	14.37	13.41	19.21	30.86	21.67	23.01	28.80
58. Picodynastornithes	49.66	35.44	33.20	36.25	57.95	53.44	56.97	54.34
59. Pici	32.52	21.22	16.79	18.94	37.95	32.00	28.81	28.39
60. Alcedinidae/Coracioidea	43.83	31.11	28.05	29.20	51.15	46.91	48.13	43.77
61. Eufalconimorphae	56.46	45.54	40.97	44.28	65.89	68.67	70.30	66.38
62. Falconidae	38.40	26.84	19.46	24.97	44.81	40.47	33.39	37.43
63. Psittacopasserae	55.49	44.69	38.07	43.07	64.76	67.39	65.33	64.56
64. Psittaciformes	30.11	17.98	17.75	15.85	35.14	27.11	30.46	23.76
65. Passeriformes	49.48	38.10	31.62	34.74	57.74	57.45	54.26	52.08
66. Eupasseres	46.98	35.38	27.43	31.47	54.83	53.35	47.07	47.17
67. Tyranni	37.25	26.61	18.81	24.29	43.47	40.13	32.28	36.41
68. Passeri	35.84	26.58	21.49	25.29	41.83	40.08	36.88	37.91
69. *Corvus*/other Passeri	28.61	20.06	16.47	19.73	33.39	30.25	28.26	29.58
70. *Sylvia*/*Vidua* + *Fringilla*	23.43	15.53	12.66	15.10	27.34	23.42	21.72	22.63
71. *Vidua*/*Fringilla*	15.50	8.75	7.65	10.91	18.09	13.20	13.13	16.35

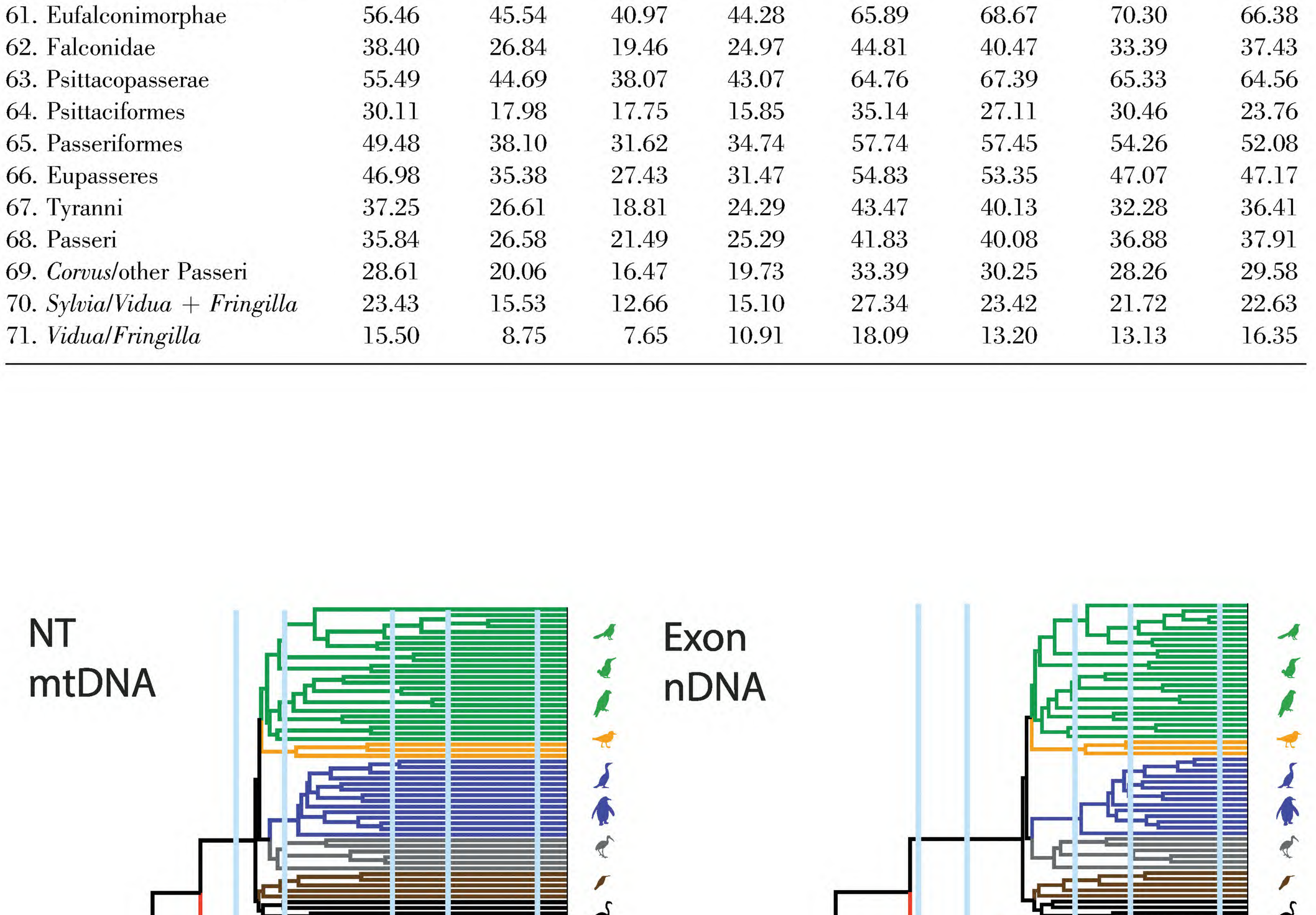


Figure 4. Nominally calibrated timetrees from divergence dating analyses in BEAST with topology constrained and only the root calibration applied. Molecular datasets correspond to those in Figures 3 and 5.

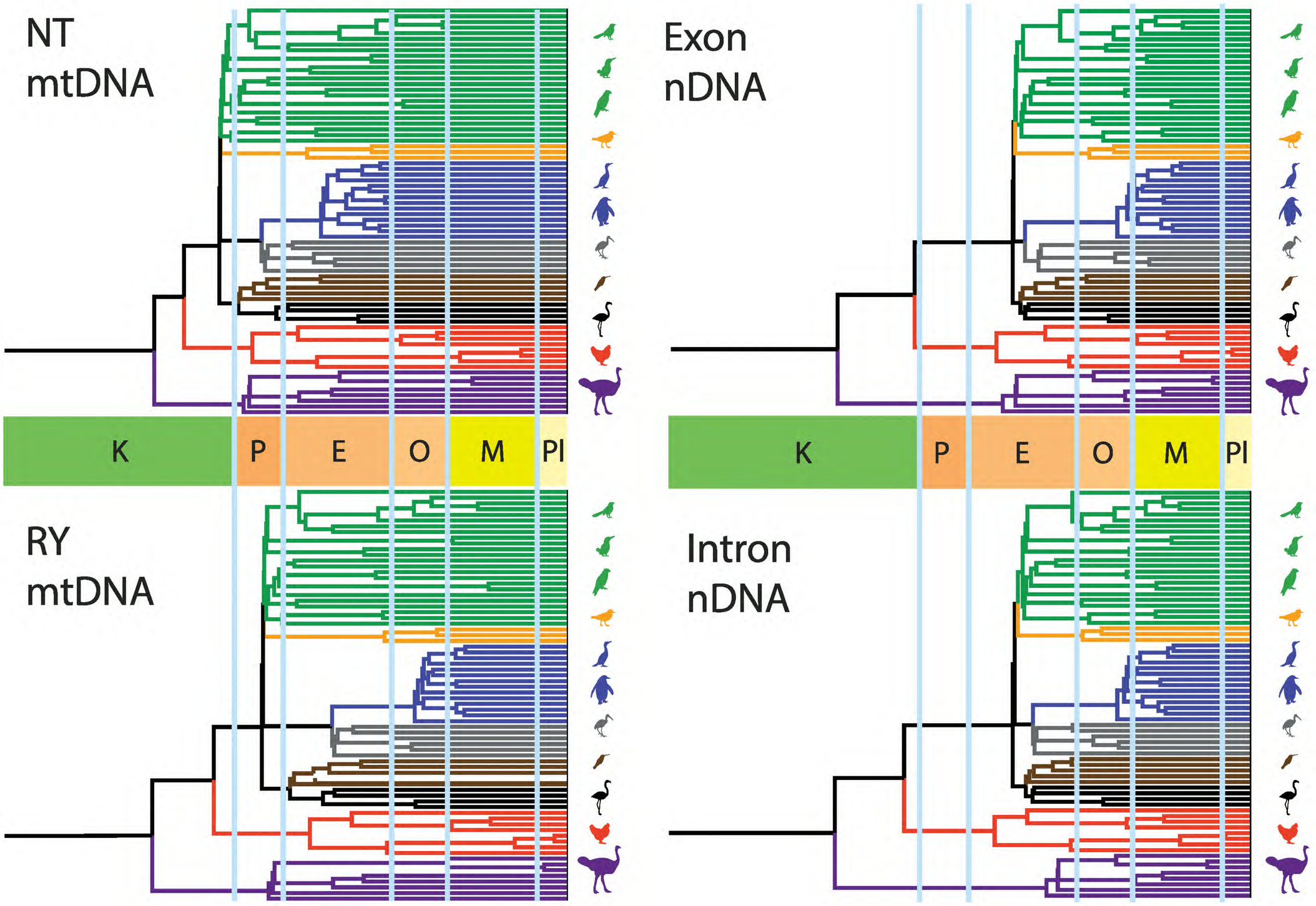


Figure 5. Timetrees from strict clock analyses. Molecular datasets correspond to those in Figures 3 and 4.

Table 4. Median divergence age estimates (Ma) for all 71 nodes from strict clock calibrated divergence dating analyses. Node numbers correspond to those seen in Figure 2.

	Strict Clock				Scaled Strict Clock			
	Mitochondrial		Nuclear		Mitochondrial		Nuclear	
Node	NT	RY	Exon	Intron	NT	RY	Exon	Intron
1. Aves	80.83	81.01	80.99	81.35	83.82	97.54	124.64	126.17
2. Palaeognathae	63.31	58.30	48.60	39.93	65.65	70.19	74.80	61.93
3. Notopalaeognathae	62.25	57.49	46.13	37.74	64.55	69.22	70.99	58.53
4. Tinamiformes/Novaeratitae	62.08	57.15	45.89	37.59	64.38	68.81	70.62	58.30
5. Novaeratitae	44.51	23.50	30.78	26.32	46.16	28.29	47.37	40.82
6. Casuariiformes	18.54	4.52	7.37	8.50	19.23	5.44	11.34	13.18
7. Tinamiformes	52.47	45.72	24.62	22.52	54.41	55.05	37.89	34.93
8. *Crypturellus/Tinamus*	46.06	39.76	17.16	17.16	47.76	47.87	26.41	26.62
9. Neognathae	75.02	69.01	65.86	67.81	77.80	83.09	101.36	105.17
10. Galloanserae	61.64	50.24	49.87	50.09	63.92	60.49	76.75	77.69
11. Galliformes	52.71	42.07	40.30	43.72	54.66	50.65	62.02	67.81
12. Numididae/Phasianidae	32.59	22.62	22.30	24.28	33.80	27.23	34.32	37.66
13. *Gallus/Coturnix*	25.48	15.01	16.27	16.94	26.42	18.07	25.04	26.27
14. Anatoidea	48.98	35.21	35.56	43.72	50.79	42.39	54.73	67.81
15. Anserinae/other Anatidae	20.96	10.29	13.47	24.28	21.74	12.39	20.73	37.66
16. *Aythya/Anas*	9.03	2.52	3.55	16.94	9.36	3.03	5.46	26.27
17. Neoaves	68.11	59.59	46.67	46.55	70.63	71.75	71.83	72.20
18. Unnamed clade	64.39	54.10	45.13	43.97	66.77	65.14	69.46	68.20
19. Mirandornithes/other birds	56.92	47.72	44.20	42.66	59.03	57.45	68.02	66.17
20. Mirandornithes	40.87	29.63	27.80	28.84	42.38	35.67	42.78	44.73
21. Columbiformes/Phaethontiformes	54.67	45.47	43.53	42.37	56.69	54.75	66.99	65.72
22. Eurypygiformes/Strisores	63.96	53.35	44.71	43.28	66.33	64.23	68.81	67.13
23. Nyctibiidae/Daedalornithes	61.33	49.56	42.18	42.43	63.60	59.67	64.92	65.81

Table 4. Continued.

Node	Strict Clock				Scaled Strict Clock			
	Mitochondrial		Nuclear		Mitochondrial		Nuclear	
	NT	RY	Exon	Intron	NT	RY	Exon	Intron
24. Daedalornithes	58.73	46.46	37.97	38.71	60.90	55.94	58.44	60.04
25. Apodiformes	48.25	40.24	32.17	35.02	50.04	48.45	49.51	54.32
26. Unnamed clade	67.95	59.37	46.48	46.15	70.46	71.48	71.53	71.58
27. Aequornithes/other birds	59.80	45.91	44.27	42.59	62.01	55.28	68.13	66.06
28. Otidiformes/other birds	59.17	45.48	41.61	42.30	61.36	54.76	64.04	65.61
29. Cuculiformes/Gruiformes	58.59	44.79	41.11	41.92	60.76	53.93	63.27	65.02
30. Cuculiformes	49.58	34.18	26.59	30.80	51.41	41.15	40.92	47.77
31. *Geococcyx/Crotophaga*	45.48	30.38	19.87	25.84	47.16	36.58	30.58	40.08
32. Grues/Rallidae	53.70	41.78	33.80	34.80	55.69	50.30	52.02	53.97
33. Aequornithes	48.21	29.81	26.14	26.07	49.99	35.89	40.23	40.43
34. Austrodyptornithes/other Aequornithes	47.73	29.17	24.19	24.76	49.50	35.12	37.23	38.40
35. Austrodyptornithes	44.76	28.12	22.10	21.13	46.42	33.86	34.01	32.77
36. Procellariiformes	39.68	21.68	17.54	18.02	41.15	26.10	26.99	27.95
37. *Puffinus/Diomedea*	36.78	20.10	15.28	16.67	38.14	24.20	23.52	25.86
38. *Ciconia*/other Aequornithes	43.25	28.28	23.79	24.21	44.85	34.05	36.61	37.55
39. Suliformes	44.87	26.99	20.19	21.63	46.53	32.50	31.07	33.55
40. Sulidae/Phalacrocoracoidea	40.10	21.81	15.37	16.19	41.58	26.26	23.65	25.11
41. Phalacrocoracoidea	36.57	19.92	13.68	15.07	37.92	23.98	21.05	23.37
42. Suliformes/Pelecaniformes	46.96	28.03	22.84	23.75	48.70	33.75	35.15	36.84
43. Pelecaniformes	41.95	21.17	22.26	22.81	43.50	25.49	34.26	35.38
44. Ardeidae/Threskiornithidae	38.65	26.23	21.20	22.28	40.08	31.58	32.63	34.56
45. *Pelecanus/Balaeniceps*	35.62	21.65	17.03	17.63	36.94	26.07	26.21	27.34
46. Litoritelluraves	67.89	59.25	46.18	45.52	70.40	71.34	71.07	70.60
47. Charadriiformes	50.88	35.69	31.63	32.86	52.76	42.97	48.68	50.97
48. *Arenaria/Larus*	38.26	25.46	26.78	29.15	39.68	30.65	41.21	45.21
49. Telluraves	67.78	59.16	46.00	44.63	70.29	71.23	70.79	69.22
50. Cathartidae/Accipitres	65.73	55.65	28.89	31.59	68.16	67.00	44.46	49.00
51. *Buteo/Pandion*	67.89	36.01	17.32	18.60	70.40	43.36	26.66	28.85
52. Afroaves	67.61	58.76	44.94	44.32	70.11	70.75	69.16	68.74
53. Strigiformes	60.66	52.78	30.92	33.03	62.90	63.55	47.59	51.23
54. Strigiformes/Eucavitaves	67.28	58.42	44.70	44.18	69.77	70.34	68.79	68.52
55. Eucavitaves	65.64	57.20	44.30	43.89	68.07	68.87	68.18	68.07
56. Picocoraciae	64.08	56.29	43.12	43.01	66.45	67.77	66.36	66.71
57. Bucerotiformes	32.05	15.37	15.53	18.41	33.24	18.51	23.90	28.55
58. Picodynastornithes	63.23	55.52	42.67	41.94	65.57	66.85	65.67	65.05
59. Pici	47.09	37.37	15.53	24.26	48.83	44.99	23.90	37.63
60. Alcedinidae/Coracioidea	58.50	53.26	39.61	38.06	60.66	64.13	60.96	59.03
61. Eufalconimorphae	66.64	58.79	45.77	44.03	69.11	70.78	70.44	68.29
62. Falconidae	50.27	39.61	23.35	23.70	52.13	47.69	35.94	36.76
63. Psittacopasserae	66.39	58.57	44.62	43.67	68.85	70.52	68.67	67.73
64. Psittaciformes	38.32	22.37	20.36	15.45	39.74	26.93	31.33	23.96
65. Passeriformes	62.28	52.34	37.35	34.90	64.58	63.02	57.48	54.13
66. Eupasseres	60.95	51.07	34.21	33.06	63.21	61.49	52.65	51.28
67. Tyranni	54.54	45.76	28.33	29.04	56.56	55.10	43.60	45.04
68. Passeri	44.56	36.90	26.87	26.62	46.21	44.43	41.35	41.29
69. *Corvus*/other Passeri	37.34	29.93	21.47	20.49	38.72	36.04	33.04	31.78
70. *Sylvia/Vidua* + *Fringilla_Passer*	33.47	26.50	18.60	17.10	34.71	31.91	28.63	26.52
71. *Vidua/Fringilla_Passer*	24.40	17.47	13.30	13.88	25.30	21.03	20.47	21.53

SIMULATION

The influence of tree compression and tree extension on divergence time estimates in otherwise controlled analyses was exemplified with data simulated on the fully calibrated exon nDNA timetree, pruned to 10 taxa, *Rhea* Brisson, *Dromaius* Vieillot, *Casuarius* Brisson, Megapodidae, *Coturnix* Garsault, *Gallus* Brisson, *Fringilla* L., *Vidua* Cuvier, *Eudyptula* Bonap., and *Diomedea* L. Seq-Gen 1.3.2 (Rambaut & Grassly, 1997) was used to simulate on the GTR+G model inferred for the original exon nDNA data, with branch lengths kept proportional to time and maintaining the overall tree length (in substitutions) for that 10-taxon subtree. Simulating under a strict clock for 20,000 nucleotide sites eliminates rate variation model errors as well as stochastic artifacts. Maximum likelihood (ML) analysis within PAUP* 4.0b10 (Swofford, 2002) under the simulated model accurately reconstructed the exon nDNA timetree.

As a simple means to producing tree compression and tree extension, additional ML reconstructions misspecified the shape of the gamma parameter (alpha). The value of alpha inferred from the exon nDNA was 0.32. This was increased to 2.0 to undercorrect for multiple substitutions at sites (saturation), resulting in tree compression. Alpha was reduced to 0.1 to overcorrect for multiple substitutions, resulting in tree extension. PAUP trees were uncalibrated, and so to compare differences in relative node heights between the treatments, each was scaled against the original 10-taxon exon nDNA tree to zero the sum of positive and negative node age differences.

Results

DIVERGENCE AGES

Analyses of the fully calibrated mtDNA and nDNA datasets all support a similar general pattern of survivorship across the K-Pg boundary (Figs. 2, 3, Table 2). Between 16 and 20 lineages among the sampled taxa are inferred to have diverged during the Cretaceous based on the median posterior node ages, though it is important to note two caveats. First, the 95% posterior probability range for most of these divergences overlaps the K-Pg boundary. Only six to eight lineages have highest posterior density (HPD) intervals that fall entirely within the Cretaceous. Second, and conversely, the number of K-Pg boundary-crossing lineages observed should be taken as a minimum estimate, as lineage birth–death processes would imply the existence of additional now-extinct lineages crossing as well.

The nominally calibrated analyses resulted in younger raw dates across the tree for all four molecular datasets (NT mitochondrial, RY mitochondrial, nuclear exons, and nuclear introns; Table 3, Fig. 4), with only three avian lineages (Palaeognathae, Galloanserae, and Neoaves) inferred to cross the K-Pg boundary. However, this is likely due to the combined influence of the single calibrated node and rate variation errors that the additional calibrations act to remedy. Scaling the nominally calibrated tree to minimize the sum of node height differences to the fully calibrated trees results in older node ages throughout the tree for all four molecular datasets (Table 3). This results in a similar number of lineages (16 to 18) inferred to have diverged during the Cretaceous as in the fully calibrated analyses.

Application of a strict clock model resulted in a modest shift relative to the fully calibrated dates for the NT mitochondrial dataset, with the average node age shifting by 7.98 Ma and 61% of node age estimates decreasing (Fig. 5). Progressively larger and more strongly negative shifts were noted in the RY mtDNA (13.68 Ma average shift, 86% decreasing), exon nDNA (20.74 Ma average shift, all decreasing), and intron nDNA datasets (21.14 Ma average shift, all but one decreasing). As with the nominally calibrated trees, scaling results in older node ages throughout the tree for all four molecular datasets (Tables 4, 5).

CODING, GENE TYPE, AND CALIBRATION STRATEGY COMPARISONS

Fully calibrated standard (NT) mtDNA data yielded significantly older ages on average for shallow nodes (nodes with median divergence ages from exon nDNA less than 66 Ma) than nuclear data ($P < 0.001$ for 2-tailed paired-sample *t*-test, though note these values may overstate significance due to non-independence of node age) (Fig. 6, Tables 2–4). Conversely, for deeper nodes (median divergence ages from exon nDNA > 66 Ma), the reverse was true and nuclear data yielded older ages than mitochondrial data ($P < 0.001$ for 2-tailed paired-sample *t*-test). These patterns are even more strongly expressed in the comparisons between the scaled nominally calibrated trees. Relatively little difference was noted between the dates from the exon nDNA and intron nDNA datasets for most nodes (Fig. 6).

Nucleotide coding regime for the mtDNA had a notable effect on divergence age estimates as well (Fig. 6). For shallow nodes (median divergence ages from RY mtDNA < 66 Ma), the NT-coded mtDNA dates were significantly older than those from the

Table 5. Number of avian lineages crossing the K-Pg boundary in the results of each analysis. The four molecular datasets include sequences for both NT- and RY-coded (A, G → R, C, T → Y) mitochondrial DNA (mtDNA) as well as translated exon and untranslated intron and nuclear DNA (nDNA).

Gene sequence datasets	Calibrations	Median posterior age Cretaceous	95% Posterior age distribution fully within Cretaceous
Exon nDNA + RY mtDNA	full	19	7
NT mtDNA	full	16	7
NT mtDNA	nominal	3	2
NT mtDNA	scaled nominal	16	—
NT mtDNA	strict clock	12	2
NT mtDNA	scaled strict clock	17	—
RY mtDNA	full	16	6
RY mtDNA	nominal	3	2
RY mtDNA	scaled nominal	17	—
RY mtDNA	strict clock	3	2
RY mtDNA	scaled strict clock	18	—
Exon nDNA	full	20	8
Exon nDNA	nominal	3	2
Exon nDNA	scaled nominal	18	—
Exon nDNA	strict clock	3	2
Exon nDNA	scaled strict clock	22	—
Intron nDNA	full	20	8
Intron nDNA	nominal	3	2
Intron nDNA	scaled nominal	18	—
Intron nDNA	strict clock	3	2
Intron nDNA	scaled strict clock	20	—

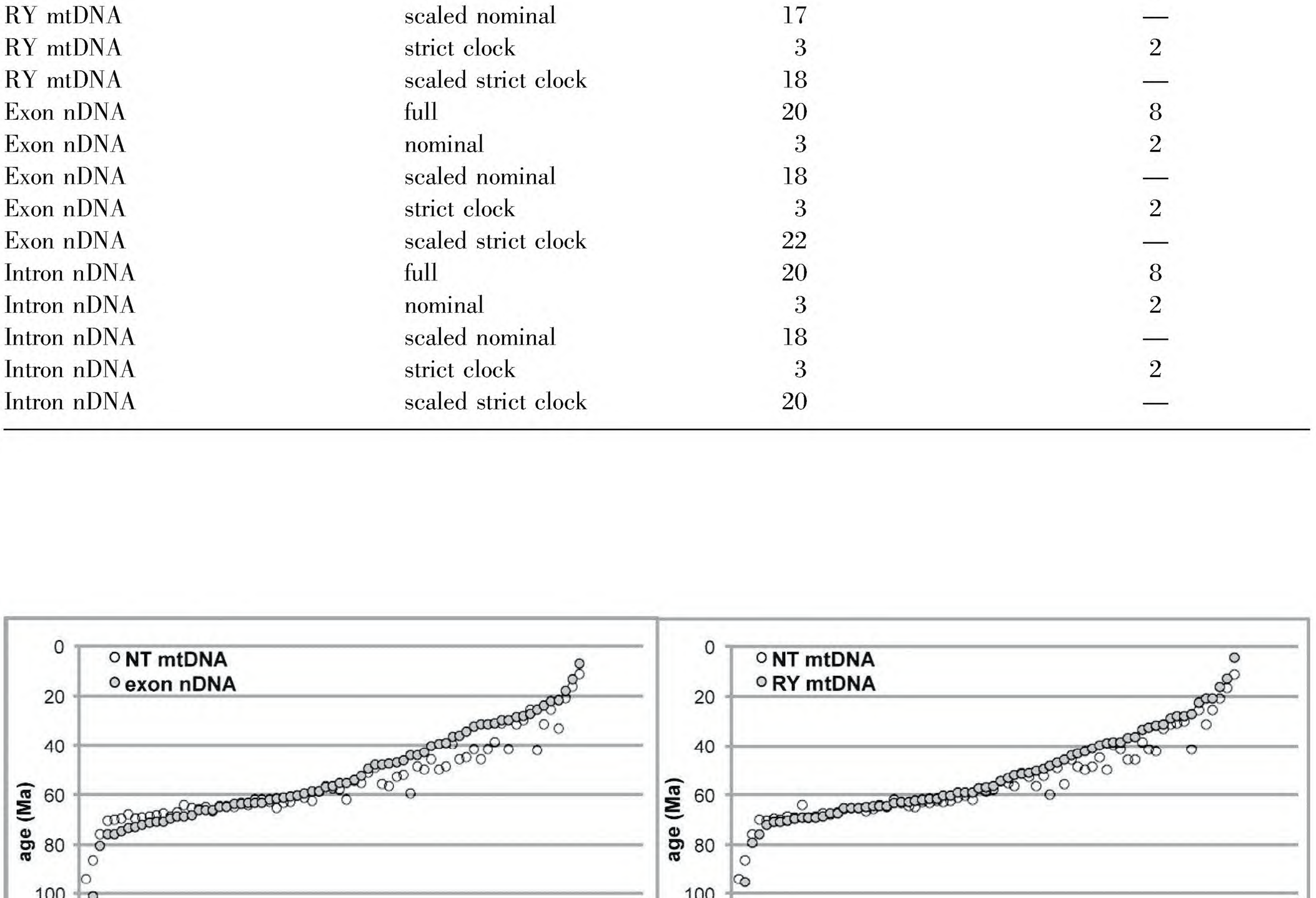


Figure 6. Comparisons of divergence estimates for all 71 nodes from analyses of NT-coded mtDNA, RY-coded mtDNA, exon nDNA, and intron nDNA. All graphs are from the analyses using the full fossil calibration set except for the bottom left, which is based on scaled, nominally calibrated analyses.

Table 6. Minimum sum of node height differences (Ma) between fully calibrated and scaled nominally calibrated trees. The four molecular datasets include sequences for both NT- and RY-coded (A, G → R, C, T → Y) mitochondrial DNA (mtDNA) as well as translated exon and untranslated intron nuclear DNA (nDNA).

Comparison	NT mtDNA	RY mtDNA	Exon nDNA	Intron nDNA
Fully calibrated to scaled nominally calibrated	169.5	406.2	401.1	294.7
Fully calibrated to scaled strict clock	550.9	761.4	573.4	609.9
Proportion of fully calibrated correction achieved by the relaxed clock model alone	0.69	0.47	0.30	0.52

RY-coded dataset ($P < 0.001$ for 2-tailed paired-sample *t*-test). Conversely, for deeper nodes (median divergence ages from RY mtDNA > 66 Ma), ages from NT-coded mtDNA were significantly younger than those from the RY-coded dataset ($P = 0.011$ for 2-tailed paired-sample *t*-test). Average difference between node age estimates is indeed greater between the RY mtDNA and NT mtDNA datasets than between the RY mtDNA and either the exon or intron nDNA dataset.

Average node age differences between fully and scaled nominally calibrated trees were lowest for the NT mtDNA dataset (Table 6). This indicates that the addition of the fossil calibrations results in more correction to the nDNA dates and the RY mtDNA dates than to the NT mtDNA dates. The overall proportion of correction from the strict clock offered by the calibrations compared to that obtained by relaxing the clock varies substantially. Relaxing the clock alone provides 69% of the overall fully calibrated correction for NT mtDNA but as little as 30% for the exon nDNA. The latter proportion is more similar to the 20% contribution of the relaxed clock model alone to correcting strict clock dates for (primarily) nuclear exon data among mammals (Phillips, in press).

A correlation between body mass and apparent dating error was detected. In each case but one, we reject the null hypothesis of no correlation between these variables at the 0.05 confidence level. No significant correlation was found for the NT mtDNA dataset. Body mass showed a significant correlation to apparent dating error in comparisons across all nodes representing pairs of tip taxa for the RY mtDNA, exon nDNA, and intron nDNA datasets (Fig. 7), with a tendency toward underestimation of node ages for larger-bodied clades.

Discussion

Patterns of disparity between datasets

A pronounced trend toward older dates in mitochondrial studies relative to nuclear studies has

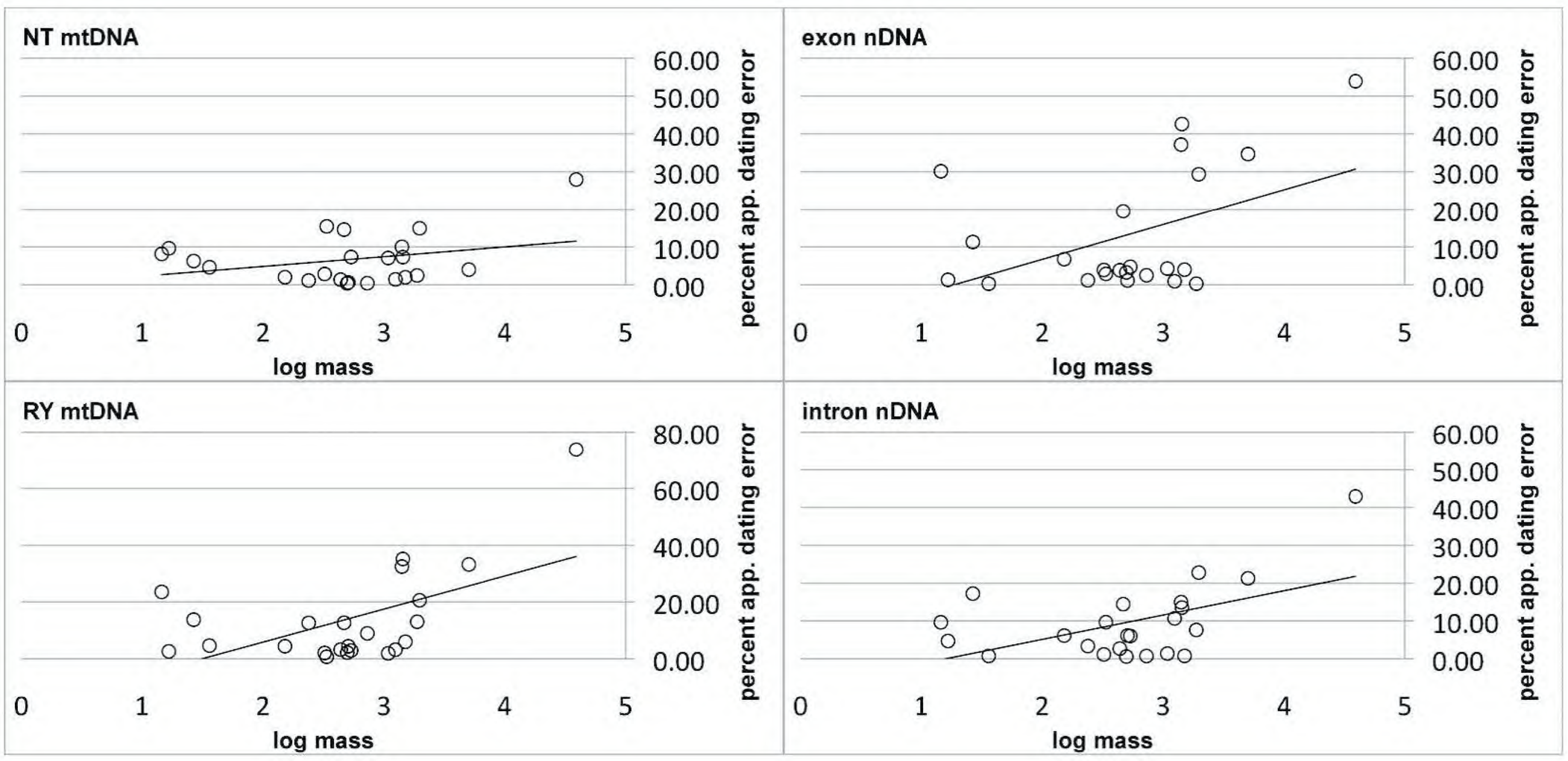


Figure 7. Apparent dating error (percentage node age difference between the fully calibrated tree and scaled nominally calibrated tree) as a function of log body mass for avian exemplars (Appendix 3). Each circle represents the node leading to a pair of tip taxa. NT mtDNA ($r^2 = 0.0966$, $P = 0.06758$), RY mtDNA ($r^2 = 0.3022$, $P = 0.003291$), exon nDNA ($r^2 = 0.2067$, $P = 0.01463$), and intron nDNA ($r^2 = 0.2714$, $P = 0.005398$) datasets.

Table 7. Extension-compression metrics for the fully calibrated relaxed clock trees. Correlation between the timetrees was tested for the difference between their paired node ages versus the average of those paired node ages. Corrected stemminess was estimated from the posterior distribution of branch lengths (substitutions.site^{-1}) in the BEAST analyses.

A. Timetree paired node age comparisons

Datasets	Spearman's rank correlation P value	Relatively extended component
NT mtDNA vs. RY mtDNA	< 0.0001	RY mtDNA
NT mtDNA vs. intron nDNA	< 0.0001	intron nDNA
NT mtDNA vs. exon nDNA	< 0.0001	exon nDNA
RY mtDNA vs. intron nDNA	$= 0.0701$	intron nDNA (not significant)
RY mtDNA vs. exon nDNA	< 0.0001	exon nDNA
Intron nDNA vs. exon nDNA	$= 0.0013$	exon nDNA

B. Corrected stemminess

	Mean	95% HPD
NT mtDNA	0.1459	0.1400–0.1518
RY mtDNA	0.2017	0.1903–0.2127
Intron nDNA	0.2336	0.2299–0.2373
Exon nDNA	0.2586	0.2493–0.2681

Abbreviations: HPD, Highest posterior density intervals; mtDNA, mitochondrial DNA; nDNA, nuclear DNA.

been observed in empirical comparisons of published avian divergence dates in one recent survey study (Ksepka et al., 2014). We observed older dates from analyses of NT mtDNA than from nDNA analyses for the majority of shallow nodes across the avian tree in the present study. However, deeper nodes exhibit the reverse pattern in our results. Because the 2014 survey study focused only on stem and crown divergences between ordinal level clades, it did not sample some of the very deep divergences, such as the Galloanserae–Neoaves split (Fig. 2, node 9), where older nuclear estimates prevail. Thus, the current results are consistent with broader patterns in the literature when sampling is taken into account.

Surprisingly, average node age differences between fully and scaled nominally calibrated trees are lowest for the NT mtDNA dataset (Table 6). This indicates that the addition of the 15 fossil calibrations exerts a larger degree of correction on the nDNA trees and the RY-coded mtDNA tree than on the NT-coded mtDNA tree. This is unexpected, as it is often presumed that standard mitochondrial data would be the most prone to branch length estimation error due to factors such as higher substitution rates and greater rate heterogeneity (Springer et al., 2001; Morgan et al., 2014). There are several possible explanations. The possibility cannot be discounted that the NT-coded mtDNA timetrees are the most accurate; the patterns of disparity with the other timetrees are consistent with tree extension operating in the nuclear and RY-coded mtDNA datasets. Alternatively, the relatively conservative maximum bounds of the calibration priors may be insufficiently informative to correct tree compression in the NT-coded mitochondrial dataset. A combination of these factors is also possible. If tree compression or extension underpins variation among timetrees, it is expected that the pairwise node age differences will depend on phylogenetic depth. Parametric tests make strong assumptions about the distribution of variation, and we instead used the non-parametric Spearman's rank correlation test. As shown in Table 7, correlations were highly significant ($P < 0.01$) for all comparisons between the four fully calibrated relaxed clock timetrees, except between the RY-coded mtDNA and the intron nDNA trees. A better indicator of relative tree compression/extension is the maximum likelihood (or Bayesian inference) corrected stemminess metric (Phillips & Penny, 2003), which is the proportion of overall tree length on internal branches. Stemminess has the statistical advantage of being based on independent branch lengths rather than potentially co-varying node ages. Tree compression and extension result in lower and higher stemminess, respectively. Corrected stemminess values (Table 7) are consistent with the timetree paired node age Spearman's rank correlation test. The pattern they confirm is that, relative to the other timetrees, the nuclear exon tree is the most extended (e.g., deep nodes relatively old, shallow nodes relatively young) and the NT-coded mtDNA tree is the most compressed (e.g., deep nodes relatively young, shallow nodes relatively old).

Life history variables potentially represent another important factor. Traits such as body mass and longevity may be strongly correlated to substitution rate and thus represent an important target for consideration in divergence dating studies. In mammals, the low substitution rate in large-bodied,

long-lived taxa relative to small-bodied, short-lived taxa has the potential to make a strong negative impact on the accuracy of inferred dates (Welch et al., 2008; Bromham, 2011; Lartillot & Delsuc, 2012; Phillips, 2015). Birds exhibit a less expansive range of body sizes (four orders of magnitude) than observed for mammals (eight orders of magnitude). Thus, body mass effects are predicted to be less influential on divergence dating results for birds. Our results suggest that body mass is correlated to apparent dating error, though to a lesser degree than observed in mammals. An important consideration here is that studies on mammals have suggested that longevity may be the driver of the relationship with evolutionary rate (Nabholz et al., 2008; Welch et al., 2008). The correlation between body size and evolutionary rate may in part be confounded by the correlation of body size with longevity. If body size is a poorer proxy for longevity in birds, then more data on longevity (and more calibrations) may eventually reveal a stronger relationship to apparent dating error than would be exposed by calibrating relaxed clocks. Likewise, future work may expand on this topic by comparing apparent dating error and life history variables across all nodes and by comparing calibrated nodes (where the correcting effect of calibrations is expected to be strongest) to uncalibrated nodes. We did not attempt comparisons sampling all nodes in the present study; this would require estimating ancestral body masses for nodes deeper in the phylogeny and the accuracy of such estimates would likely suffer greatly from lack of taxonomic sampling (e.g., approximately 1% of all living species).

Our results reaffirm the importance of multiple calibrations in informing clock models when dealing with heterogeneous rates (Waddell et al., 2001; Yang & Rannala, 2006). For example, the Sphenisciformes–Procellariiformes (penguin–petrel) split (Fig. 2, node 35) is recovered as 25.90–47.55 Ma in the nominally calibrated trees (44.44–55.49 Ma in the scaled nominally calibrated trees), whereas the fully calibrated tree yields substantially older dates (62.11–63.27 Ma) due to a 61 Ma minimum fossil calibration based on the oldest reported fossil penguin. Beyond this single calibrating fossil, thousands of older Paleogene penguin specimens are known from throughout the Southern Hemisphere, leaving no doubt that the uncalibrated divergence date is a severe underestimate. Because penguins and many procellariiforms are relatively long-lived, large-bodied birds, this may seem a case of life history traits influencing inferred substitution rates. The associated possibility of parallel rate deceleration in both lineages would provide a particularly difficult scenario for current rate models. However, deeper investigations are warranted before ascribing this explanation, as high levels of apparent age underestimation also can be detected at a few nodes that represent smaller-bodied clades. For example, the Apodidae–Trochilidae (hummingbird–swift) split (Fig. 2, node 25) represents the divergence between two of the smallest-bodied avian clades and is recovered as 20.23–40.39 Ma in age in the nominally calibrated trees (34.71–47.14 Ma in the scaled nominally calibrated trees). The fully calibrated tree yields older dates (49.68–51.72 Ma).

AVIAN EVOLUTION AND THE K-PG BOUNDARY

Our favored analyses (fully calibrated RY-mtDNA + exon nDNA dataset) support substantially younger ages for most interordinal divergences compared to many previous studies. However, the new dates do not resolve whether the early radiation of crown birds was a primarily Cretaceous versus Paleogene event due to the extension of the 95% HPD intervals for many divergences across the K-Pg boundary (tree file available at Dryad, <doi:10.5061/dryad.3c3r3>). One intractable issue in Bayesian divergence dating methods is that, due to the confounding effect of rate and time, posterior estimates of divergence time would retain some uncertainty even if an infinite amount of sequence data were available (dos Reis & Yang, 2013). Thus, currently favored divergence dating methods may lack the ability to distinguish a rapid Early Paleocene radiation from a Late Cretaceous radiation unless unreasonable strict priors are enforced, which would essentially answer the question before the analysis was initiated. Despite these limitations, it is worthwhile to test alternate scenarios in other ways. One simple test of the Paleogene radiation scenario is to assess whether implausible rates of evolution are required by a given set of assumptions. In the case of placental mammals, a recently articulated scenario of the explosive Early Paleocene radiation (O'Leary et al., 2013) was challenged on the basis that a large number of interordinal divergences occurring within 200 thousand years (Ka) of the Paleocene would require "virus-like" substitution rates (ca. 0.2 substitutions per nucleotide per million years) (Springer et al., 2013: 613). In the case of crown birds, it is possible to show that an explosive model does not require unreasonable assumptions regarding rates of sequence evolution. When priors are enforced so that all divergences except the Palaeognathae–Neognathae, Galloanserae–Neoaves, and Galliformes–Anseriformes splits are constrained to occur after the K-Pg

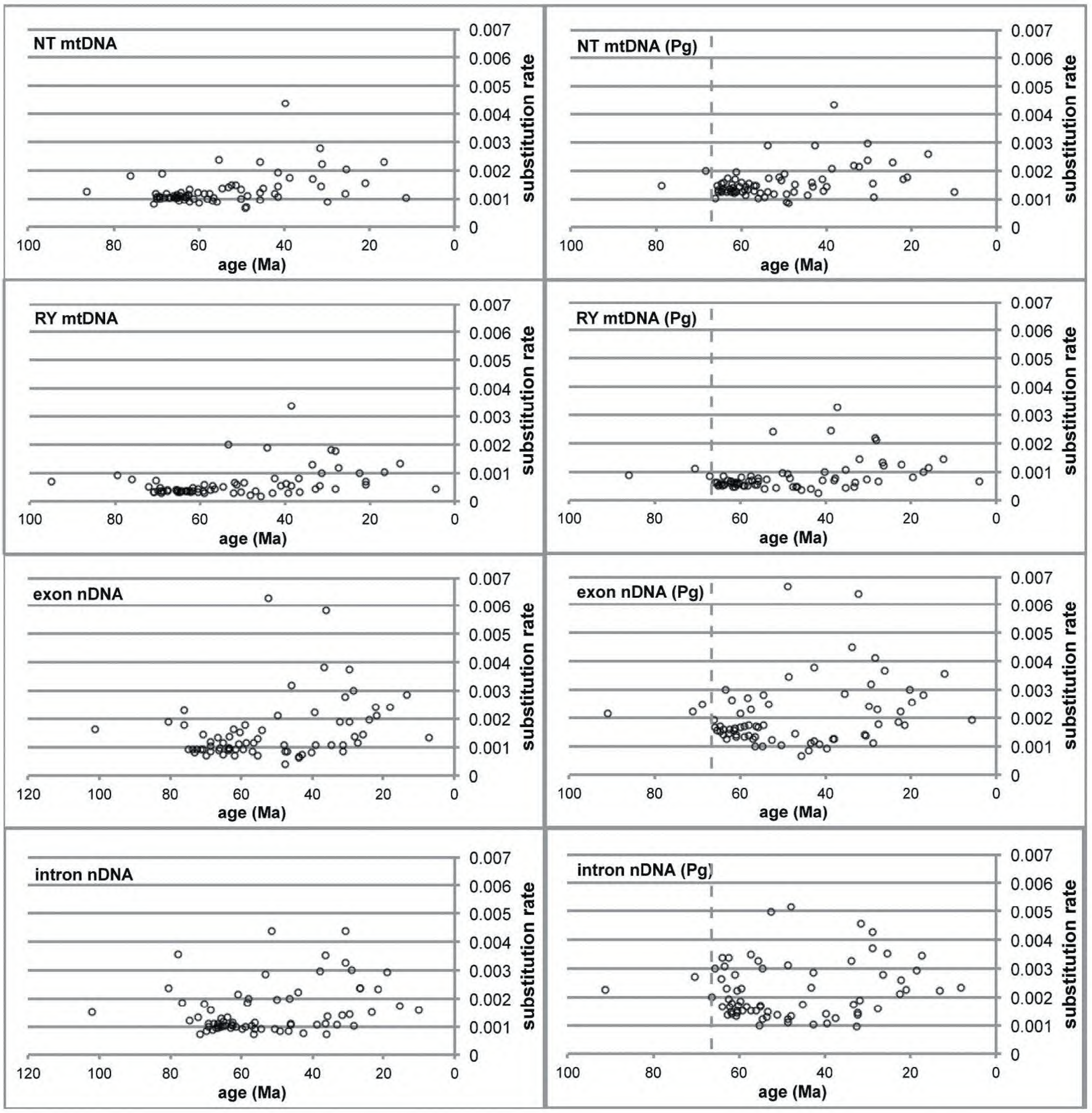


Figure 8. Inferred substitution rates (substitutions per site per million years) along branches leading to each dated node from fully calibrated analyses. Right-side graphs are from analyses in which additional maximum constraints were applied so that all divergences except those for the Palaeognathae, Neognathae, and Galloanserae are constrained to occur after the K-Pg boundary (66 Ma), as indicated by "Pg" notation.

boundary, no major substitution rate increase is observed for Paleocene divergences (Fig. 8). Indeed, the average Paleocene substitution rate is actually inferred to be lower than the average post-Paleocene substitution rate.

Beyond substitution rates, ecological factors deserve careful consideration. The number of lineages passing through the K-Pg boundary is an important indicator of the severity of the Cretaceous mass extinction of birds. Many molecular studies support dozens of major lineages that cross the K-Pg boundary, which at face value would represent an impressive survival rate during a mass extinction event. However, this represents only the tip of the iceberg, as previously noted in the case of placental mammals (Phillips, in press). Most avian divergence studies do not sample taxa completely, even at the ordinal level, and so may underestimate the lineages that would otherwise be placed in the Cretaceous in their results. Beyond this, even complete molecular sampling will detect only lineages leading to extant species, whereas birth–death models would predict the presence of some proportion of dead branches leading to now-extinct clades at any given point in time. Taking such factors into account, many molecular estimates of diversity across the Creta-

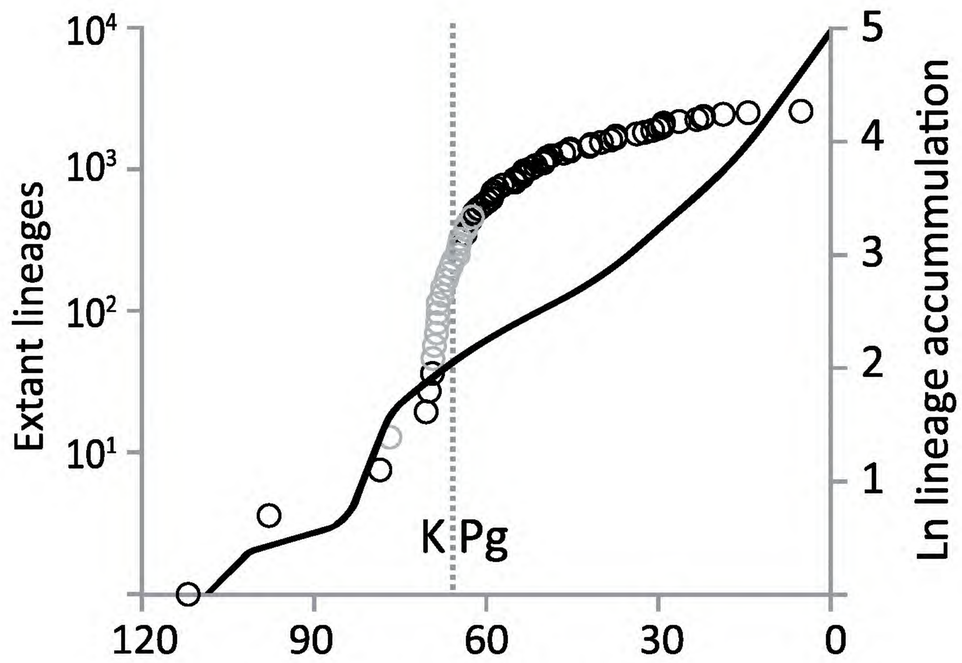


Figure 9. Lineage-through-time accumulations for Aves. Open circles represent ages inferred from the favored RY-mtDNA + exon nDNA fully calibrated relaxed clock analysis and are matched to the right scale. Gray circles indicate divergences that have 95% highest posterior density intervals that cross the K-Pg boundary. The solid line represents the lineage-through-time plot of Jetz et al. (2012) and is matched to the left scale.

ceous–Paleogene boundary are difficult to reconcile with the severity of this mass K-Pg extinction, which completely wiped out non-avian dinosaurs, pterosaurs, and previously successful archaic bird clades such as the Enantiornithes and Hesperornithes.

A related problem arises when one considers inferred diversification patterns during the Paleocene. Lineage-through-time analyses from studies supporting a major intraordinal diversification in the Cretaceous also suggest a significant diversification rate slowdown throughout the Paleocene (e.g., Jetz et al., 2012). Yet, it seems counterintuitive to posit a major slowdown in diversification rate during a time when crown birds should have been free to radiate into vacant ecological space opened up by the extinction of pterosaurs, all remaining stem bird lineages, and presumably many crown bird species. At the same time that a diversification slowdown should be occurring according to Jetz et al.'s (2012) lineage-through-time data, the fossil record records the rapid first appearances of many major lineages of birds and also supports a higher level of ecological diversity than seen in Cretaceous avifaunas (Mitchell & Makovicky, 2014). In contrast, our combined RY-coded mtDNA and exon nDNA analysis places the same explosive diversification of avian diversity near the K-Pg boundary, sufficiently close for the difference to potentially be explained by minor errors associated with tree extension and life history rate-correlations (Fig. 9).

Molecular phylogenetic analyses generally agree on the presence of a cluster of short internal branches near the base of Neoaves (e.g., Hackett et al., 2008; Pacheco et al., 2011). This cluster of short branches at the base of Neoaves (Fig. 2, node 17) is more easily reconciled with the fossil record and major events in Earth history by positing a rapid diversification in the aftermath of the Cretaceous–Paleogene extinction than a Cretaceous diversification followed by mass survival across the K-Pg boundary. Therefore, we lean toward interpreting the majority of divergences with HPD intervals overlapping the K-Pg boundary as Early Paleocene events. Given the difficulty in resolving temporal patterns in the early crown radiation of birds with molecular data only, focusing on the latest Cretaceous fossils will remain critical. Our preferred dates suggest that several lineages that represent the stems leading to multi-order clades may pass through the K-Pg boundary, and identifying fossil taxa that belong to these stem lineages will likely require both more complete specimens and more detailed morphological phylogenetic work.

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Appendix 1. Fossil calibrations used in this study.

Node Calibrated: Casuariiformes (*Dromaius* + *Casuarius*)

Fig. 2, node 6.

Prior shape. Uniform.

Fossil specimen. †*Emuarius gidju* (QM F45460).

Phylogenetic justification. Worthy et al. (2014) recovered †*Emuarius* as more closely related to *Dromaius* than *Casuarius* in a phylogenetic analysis. Codings for †*Emuarius* were based on multiple specimens, and key synapomorphies occur in the skull, tarsometatarsus, and scapulocoracoid. We have thus selected a scapulocoracoid (QM F45460) as the calibrating specimen, although it should be noted that other elements also occur at the same site.

Hard minimum age. 24.5 Ma.

Minimum age justification. QM F45460 was collected from Faunal Zone A at the Hiatus South Site of the Riversleigh locality in Queensland, Australia. Based on biocorrelation to the faunas from the Etadunna and Namba Formations in South Australia (Woodburne et al., 1994), a minimum age matching the top of Chron 7r is applied, with the numerical date selected from table 28.2 of Gradstein et al. (2012).

Soft maximum age. 58.7 Ma.

Maximum age justification. The soft maximum is based on the age of the oldest putative Palaeognathae, which include Middle–Late Paleocene lithornithids from North America (Houde, 1988) and the ratite †*Diogenornis*, from the Early Eocene of Brazil (Alvarenga, 1983). While the precise phylogenetic relationships of these taxa are debated, none are plausibly nested within crown Casuariiformes.

Node Calibrated: Tinamiformes (Nothurinae + Tinaminae)

Fig. 2, node 8.

Prior shape. Uniform.

Fossil specimen. Tinamidae sp. indet. (MACN-SC-1399).

Phylogenetic justification. A phylogenetic analysis by Bertelli and Chiappe (2005) placed MACN-SC-1399 within the crown clade Tinamidae, along the stem lineage leading to Nothurinae (aridland tinamous).

Hard minimum age. 16.9 Ma.

Minimum age justification. MACN-SC-1399 was collected from the Pinturas Formation of Argentina. The hard minimum age is based on a radiometric date from a tuff that lies on the boundary between Pinturas Formation and Santa Cruz Formation (Perkins et al., 2012).

Soft maximum age. 58.7 Ma.

Maximum age justification. The soft maximum is based on the age of the oldest putative Palaeognathae, which include Middle–Late Paleocene lithornithids from North America (Houde, 1988) and the ratite †*Diogenornis*, from the Early

Eocene of Brazil (Alvarenga, 1983). While the precise phylogenetic relationships of these taxa are debated, none are plausibly nested within the crown Tinamiformes.

Node Calibrated: Galliformes (Megapodidae + Numididae + Phasianidae)
Fig. 2, node 11.

Prior shape. Uniform.
Fossil specimen. †*Palaeortyx brevipes* (PW 2005/5023a-LS).
Phylogenetic justification. Mayr et al. (2006) described apomorphies, including the well-developed processus intermetacarpalis, that support placement of †*Palaeortyx brevipes* within crown Galliformes, as a stem representative of Phasianidae. PW 2005/5023a-LS represents a nearly complete skeleton and thus is selected as the calibrating specimen.
Hard minimum age. 24.0 Ma.
Minimum age justification. PW 2005/5023a-LS was collected from a maar lake deposit at Enspel, near Bad Marienberg in Westerwald, Rheinland-Pfalz, Germany. These deposits are assigned to the MP28 biozone (Storch et al., 1996), the top of which is used for the hard minimum age.
Soft maximum age. 51.57 Ma.
Maximum age justification. The soft maximum is based on the age of the Green River Formation from which multiple complete skeletons of the stem galliform †*Gallinuloides wyomingensis* have been collected. This maximum encompasses other strata that have yielded good material of stem galliforms but no convincing crown galliform material, including the Messel Formation, Eocene horizons at Quercy, and the London Clay Formation. Although the minimum age we specified is likely to be extended backward in time with future revisions of the galliform fossil record, the uniform prior accommodates this by encompassing the ages of taxa that may possibly represent crown galliforms but require additional study, such as †*Procrax* and †*Schaubortyx*.

Node Calibrated: Anatoidea (Anatidae + Anseranatidae)
Fig. 2, node 14.

Prior shape. Approximate lognormal.
Fossil specimen. †*Vegavis iaai* (MLP 93-I-3-1).
Phylogenetic justification. Phylogenetic analyses by Clarke et al. (2005) placed †*Vegavis* (which is known only from the holotype MLP 93-I-3-1) within Anseriformes, along the stem lineage leading to Anatidae.
Hard minimum age. 66 Ma.
Minimum age justification. MLP 93-I-3-1 was collected from near the lower base of the dinoflagellate *Manumiella bertodano* biozone in lithostratigraphic unit K3, also referred to as Unit "C," or the Sandwich Bluff Member, of the Lopez de Bertodano Formation at Sandwich Bluff, Vega Island, Antarctica. The minimum age is based on the age of the *M. bertodano* biozone as well as the inferred sedimentation rate.
Soft maximum age. 86.3 Ma.
Maximum age justification. The soft maximum is based on the lower bound of the Santonian stage. This accounts for the presence of numerous stem birds and absence of crown birds of any type, let alone Anseriformes, throughout this part of the Late Cretaceous (after Benton & Donoghue, 2007).

Node Calibrated: Unnamed Clade (Phaethontiformes + Columbiformes)
Fig. 2, node 21.

Prior shape. Approximate lognormal.
Fossil specimen. †*Lithoptila abdounensis* (OCP.DEK/GE 1087).
Phylogenetic justification. Phylogenetic analyses by Bourdon et al. (2005) and Smith (2010) recovered †*Lithoptila abdounensis* as a stem representative of Phaethontidae, and cranial characters preserved in OCP.DEK/GE 1087 support this placement. Although the position of Phaethontidae within Aves is controversial, there is no doubt regarding the placement of †*Lithoptila*, which tracks Phaethontidae regardless of the arrangement of other taxa. Therefore, the calibration is placed at the node uniting Phaethontiformes and Columbiformes in the present study.
Hard minimum age. 56.0 Ma.
Minimum age justification. OCP.DEK/GE 1087 was collected from an unspecified quarry, assigned to Bed IIa of the Ouled Abdoun Basin, near Grand Daoui, Morocco, which in turn can be assigned to the Thanetian based on selachians identified in the matrix (Bourdon et al., 2005). As both the precise numerical age of Bed IIa deposits and the precise horizon from which the *Lithoptila abdounensis* holotype was collected remain uncertain, the lower age bound for the Thanetian is used as a hard minimum.
Soft maximum age. 72.1 Ma.
Maximum age justification. The maximum age extends to the base of Maastrichtian to accommodate the possibility that some of the poorly represented marine birds from the Cretaceous–Paleogene of New Jersey, U.S.A., may represent tropicbirds (Bourdon et al., 2008).

Node Calibrated: Apodiformes (Apodidae + Trochilidae)
Fig. 2, node 25.

Prior shape. Uniform.
Fossil specimen. †*Scaniacypselus szarskii* (SMF-ME 11345A+B).
Phylogenetic justification. Phylogenetic analysis of morphological data (Mayr, 2003, 2005c) and combined morphological and molecular data (Ksepka et al., 2013) supports the placement of †*Scaniacypselus szarskii* as a stem member of Apodidae. Several characters of the wing are optimized as synapomorphies supporting this position, and can be observed in the partial skeleton of SMF-ME 11345A+B (Mayr, 2014b).
Hard minimum age. 47.0 Ma.
Minimum age justification. A maximum age for the fossiliferous deposits of the Messel Formation is provided by a 47.8 ± 0.2 Ma 40Ar/39Ar age obtained from the basalt chimney below Lake Messel (Mertz et al., 2004). This date provides a maximum age for Lake Messel itself, but a minimum age for the fossil must take into account time elapsed between the cooling of the basalt and the deposition of the fossiliferous layers that occur higher in the section. Lacustrine sediments are estimated to have filled in the maar lake that formed above this basalt chimney over a span of approximately 1 Ma (Mertz et al., 2004). Accounting for sedimentation rate, the layers yielding avian fossils (including SMF-ME 1883a+b) are most likely ~47 Ma in age (Mertz et al., 2004; Franzen, 2005). The Messel Formation has also yielded the stem hummingbird †*Parargornis messelensis* (Mayr, 2003), providing further support for this minimum constraint. Ksepka and Clarke (2015) recommended the slightly older †*Scaniacypselus wardi* at 51 Ma as a minimum calibration. We do not dispute this fossil's phylogenetic placement, but opted to use the slightly younger but more complete †*S. szarskii* specimen as a more conservative minimum.
Soft maximum age. 66.5 Ma.
Maximum age justification. The latest Cretaceous is set as the soft maximum, corresponding to the age range of the oldest known crown bird †*Vegavis*. No members of the nightbird–swift–hummingbird clade Strisores (to which Apodiformes

belong) are known from Cretaceous deposits, indicating it is unlikely the highly nested divergence between hummingbirds and swifts occurred before the Paleocene.

NODE CALIBRATED: AUSTRODYPTORNITHES (SPHENISCIFORMES + PROCELLARIIFORMES)
Fig. 2, node 35.

Prior shape. Approximate lognormal.

Fossil specimen. †*Waimanu manneringi* (CM zfa35).

Phylogenetic justification. Phylogenetic analysis supports the placement of †*Waimanu manneringi* along the penguin stem lineage (e.g., Ksepka et al., 2006; Slack et al., 2006). CM zfa35 is the only described specimen of †*W. manneringi*.

Hard minimum age. 60.5 Ma.

Minimum age justification. CM zfa35 was collected from the basal Waipara Greensand (Slack et al., 2006). Biostratigraphic evidence, specifically the ranges of *Hornibrookina teuriensis* and *Chaismolithus bidens*, indicates the minimum possible age of the fossil is 60.5 Ma (Cooper, 2004; Slack et al., 2006; Ogg et al., 2008).

Soft maximum age. 72.1 Ma.

Maximum age justification. The soft maximum is based on the lower bound of the Maastrichtian stage (Late Cretaceous). Southern Hemisphere Maastrichtian marine vertebrate sites have yielded diving birds such as †*Polarornis* and hesperornithids, indicating preservation potential for marine diving birds, but no penguin (or procellariiform) remains have been recovered at these sites.

NODE CALIBRATED: SULIFORMES (FREGATIDAE + SULIDAE + PHALACROCORACOIDEA)
Fig. 2, node 39.

Prior shape. Approximate lognormal.

Fossil specimen. †*Limnofregata azygosternon* (USNM 22753).

Phylogenetic justification. Phylogenetic analysis supports the placement of †*Limnofregata* as the sister taxon to extant *Fregata* (Smith, 2010), in agreement with long-standing interpretations of this fossil taxon (Olson, 1977). USNM 22753 is an articulated skeleton preserving most key synapomorphies that place †*L. azygosternon* on the frigatebird stem lineage.

Hard minimum age. 51.57 Ma.

Minimum age justification. USNM 22753 was collected from the Fossil Butte Member of the Green River Formation in Wyoming, U.S.A. The minimum date of 51.57 Ma incorporates the error associated with an $^{40}Ar/^{39}Ar$ date of 51.66 ± 0.09 Ma obtained from a potassium-feldspar (K-spar) tuff above the fossiliferous horizon containing USNM 336484 (Smith et al., 2008).

Soft maximum age. 66.5 Ma.

Maximum age justification. The latest Cretaceous is set as the soft maximum, corresponding to the age range of the oldest known crown bird †*Vegavis*. No members of the core waterbird clade Aequornithes (to which Suliformes belong) are known from Cretaceous deposits, indicating it is unlikely the highly nested divergence between frigatebirds and other Suliformes occurred before the Paleocene.

NODE CALIBRATED: PHALACROCORACOIDEA (ANHINGIDAE + PHALACROCORACIDAE)
Fig. 2, node 41.

Prior shape. Uniform.

Fossil specimen. †*Oligocorax* (= *Borvocarbo*) *stoeffelensis* (PW 2005/5022-LS).

Phylogenetic justification. The phylogenetic analysis of Smith (2010) recovered †*Oligocorax stoeffelensis* nested within Phalacrocoracoidea and more closely related to Phalacrocoracidae than to Anhingidae, whereas a phylogenetic analysis by Mayr (2015) recovered two alternate topologies, one placing †*O. stoeffelensis* as stem member of Phalacrocoracidae and one placing this fossil taxon within crown Phalacrocoracidae. Given the uncertainty, we conservatively place this calibration at the node representing the split between Phalacrocoracidae and Anhingidae. PW 2005/5022-LS preserves a substantial portion of the skeleton, including synapomorphy-bearing elements that support placement to at least the stem lineage of Phalacrocoracidae.

Hard minimum age. 24.52 Ma.

Minimum age justification. PW 2005/5022-LS was collected from the Enspel locality at Bad Marienberg in Westerwald, Rheinland-Pfalz, Germany (Mayr, 2007). Radiometric dating of basaltic flows that bound the Enspel deposits yielded ages of 24.56 ± 0.04 Ma (Mertz et al., 2007).

Soft maximum age. 52 Ma.

Maximum age justification. The soft maximum is based on the age of the oldest reported specimens assigned to Suliformes (the clade including Phalacrocoracoidea, Sulidae, and Fregatidae), which belong to the stem frigatebird †*Limnofregata* from the Early Eocene Green River Formation. No stem or crown Sulariformes are known from beyond this date.

NODE CALIBRATED: UNNAMED CLADE (ARDEIDAE + THRESKIORNITHIDAE)
Fig. 2, node 44.

Prior shape. Approximate lognormal.

Fossil specimen. †*Rhynchaeites* sp. indet. (MGUH 20288).

Phylogenetic justification. Multiple apomorphies support the placement of †*Rhynchaeites* within the Threskiornithidae (Mayr & Bertelli, 2011). Although the characteristic ibis-type bill is not preserved in MGUH 20288, derived characteristics of the hindlimb support assignment to †*Rhynchaeites* as well as placement along the stem lineage of Threskiornithidae (Mayr & Bertelli, 2011).

Hard minimum age. 53.9 Ma.

Minimum age justification. MGUH 20288 was collected from the Fur Formation of Denmark. The minimum age is based on a 54.04 ± 0.14 Ma radiometric date reported for layer +19 of the Fur Formation (Chambers et al., 2003).

Soft maximum age. 66.5 Ma.

Maximum age justification. The latest Cretaceous is set as the soft maximum, corresponding to the age range of the oldest known crown bird †*Vegavis*. No members of the core waterbird clade Aequornithes (to which Threskiornithidae belongs) are known from Cretaceous deposits, indicating it is unlikely the highly nested divergence between ibises and herons had occurred before the Paleocene.

NODE CALIBRATED: PELECANIFORMES (PELECANIDAE + BALAENICIPITIDAE)
Fig. 2, node 45.

Prior shape. Uniform.

Fossil specimen. *Pelecanus* sp. indet. (NT-LBR-039).

Phylogenetic justification. NT-LBR-039 is essentially identical to modern pelicans in cranial osteology and preserves multiple synapomorphies of Pelecanidae (Louchart et al., 2011).

Hard minimum age. 28.1 Ma.

Minimum age justification. NT-LBR-039 was collected from the Pichovet locality in Lubéron, France (Louchart et al., 2011). The fossil horizon is Rupelian in age (Oligocene), and

thus the upper boundary of the Rupelian stage is used as the hard minimum.

Soft maximum age. 53.9 Ma.

Maximum age justification. The soft maximum age is based on the age of the oldest members of Ciconiiformes s.l. (†*Rhynchaeites*, see above) and the absence of other convincing representatives of Pelecaniformes, Sulariformes, and Ciconiiformes in deposits older than 53.9 Ma.

NODE CALIBRATED: EUCAVITAVES (TROGONIDAE + PICOCORACIAE)
Fig. 2, node 55.

Prior shape. Approximate lognormal.

Fossil specimen. †*Septentrogon madseni* (MGUH VP3496).

Phylogenetic justification. Cranial characters including the mediolaterally flattened and rounded zygomatic processes, a synapomorphy of trogons, support placement of †*Septentrogon* to at least the trogon stem lineage (Kristoffersen, 2002).

Hard minimum age. 53.9 Ma.

Minimum age justification. MGUH VP3496 was collected from the Fur Formation of Denmark. The minimum age is based on a 54.04 ± 0.14 Ma radiometric date reported for layer +19 of the Fur Formation (Chambers et al., 2003).

Soft maximum age. 66.5 Ma.

Maximum age justification. The latest Cretaceous is set as the soft maximum, corresponding to the age range of the oldest known crown bird †*Vegavis*. No members of the higher landbird clade Telluraves (to which Trogoniformes belong) are known from Cretaceous deposits, indicating it is unlikely that the nested divergence of trogons from other landbirds had occurred before the Paleocene.

NODE CALIBRATED: UNNAMED CLADE (ALCEDINIDAE + CORACIOIDEA)
Fig. 2, node 60.

Prior shape. Approximate lognormal.

Fossil specimen. †*Primobucco mcgrewi* (USNM 336484).

Phylogenetic justification. Phylogenetic analyses place †*Primobucco mcgrewi* along the stem lineage leading to the clade Coracioidea (rollers and ground rollers) (Mayr et al., 2004; Clarke et al., 2009).

Hard minimum age. 51.57 Ma.

Minimum age justification. USNM 336484 was collected from the Middle unit of the Fossil Butte Member of the Green River Formation in Wyoming, U.S.A. The minimum date of 51.57 Ma incorporates the error associated with an $^{40}Ar/^{39}Ar$ date of 51.66 ± 0.09 Ma obtained from a potassium-feldspar (K-spar) tuff above the fossiliferous horizon containing USNM 336484 (Smith et al., 2008).

Soft maximum age. 66.5 Ma.

Maximum age justification. The latest Cretaceous is set as the soft maximum, corresponding to the age range of the oldest known crown bird †*Vegavis*. No members of the higher landbird clade Telluraves (to which Coraciiformes belong) are known from Cretaceous deposits, indicating it is unlikely the highly nested roller–kingfisher divergence had occurred before the Paleocene.

NODE CALIBRATED: PSITTACOPASSERAE (PSITTACIFORMES + PASSERIFORMES)
Fig. 2, node 63.

Prior shape. Approximate lognormal.

Fossil specimen. †*Pulchrapollia gracilis* (NHMUK A6207).

Phylogenetic justification. Phylogenetic analyses have consistently recovered †*Pulchrapollia gracilis* as a stem lineage parrot (Dyke & Cooper, 2000; Mayr, 2005b; Ksepka et al., 2011).

Hard minimum age. 53.5 Ma.

Minimum age justification. NMHUK A6207 was collected from the Walton Member (Division A2) of the London Clay Formation at Walton-on-the-Naze, England. The Walton Member correlates to the upper part of Chron C24r, and the minimum age is based on the youngest estimate for the top of C24r (53.54 ± 0.04) presented by Westerhold et al. (2007). Additional evidence for this minimum date is provided by the oldest reported Passeriformes, which are more fragmentary remains from the Early Eocene Murgon site of Australia (Boles, 1995, 1997).

Soft maximum age. 66.5 Ma.

Maximum age justification. The latest Cretaceous is set as the soft maximum, corresponding to the age range of the oldest known crown bird †*Vegavis*. No members of the higher landbird clade Telluraves (to which the Psittacopasserae belongs) are known from Cretaceous deposits, indicating it is extremely unlikely the highly nested parrot–songbird divergence had occurred before the Paleocene.

NODE CALIBRATED: EUPASSERES (TYRANNI + PASSERI)
Fig. 2, node 66.

Prior shape. Uniform.

Fossil specimen. SMNS 59466/1 (suboscine sp. indet.).

Phylogenetic justification. The presence of a distally protruding fingerlike process at the cranial edge of metacarpal III is an apomorphic feature supporting assignment of SMNS 59466/1 to at least the suboscine stem lineage.

Hard minimum age. 26 Ma.

Minimum age justification. SMNS 59466/1 was collected from the Herrlingen 11 locality at Ulm, Baden-Württemberg, Germany (Manegold, 2008). Szyndlar (1994) determined an age of Mammal Paleogene (MP) zones 28 to 30 for this locality, and Manegold (2008) indicated an age of MP 28 based on a personal communication from Bötticher. Thus, the lower age limit of MP 28 is used as a hard minimum age. Additional fossils from this site have been assigned to the oscine lineage, providing additional support for this constraint.

Soft maximum age. 55.0 Ma.

Maximum age justification. The oldest reported Passeriformes are from the Early Eocene Murgon site of Australia (Boles, 1995, 1997). These fossils bear primitive characters indicating they fall outside Eupasseres (Mayr, 2013). Furthermore, no crown Passeriformes are found in Eocene deposits, such as the Green River Formation and Messel Formation, which otherwise preserve an abundance of small birds. Thus, the age of the Murgon fossils is used a soft maximum.

APPENDIX 2. Sources for sequence data. All nuclear data for primary species follow Hackett et al. (2008). GenBank accession numbers for mitochondrial genomes are listed after the taxonomic identification. Other species names and accession numbers for genes were added to composite taxa to improve sequence representation. UTRs represent untranslated regions of nuclear DNA.

Primary species for nuclear loci	Matching mt genome	Taxa sampled to improve sequence representation
Acanthisitta chloris	*Acanthisitta chloris* (AY325307)	
Aegotheles insignis	*Aegotheles cristatus* (NC_011718)	
Aerodramus vanikorensis	*Apus apus* (NC_008540)	
Alcedo leucogaster	*Todiramphus sanctus* (NC_011712)	*Momotus momota* ALDOB (EU737869), NGFB (EU740156), MUSK (EU739836), *Todus angustirostris* EGR1 [exons only] (EU739036), NTF3 (EU740377)
Alectura lathami	*Alectura lathami* (AY346091)	*Megapodius eremita* FGB [exons only] (EU739436)
Anas platyrhynchos	*Anas falcata* (KC759527)	
Anhinga anhinga	*Anhinga rufa* (NC_014681)	
Anser erythropus	*Anser albifrons* (AF363031)	
Anseranas semipalmata	*Anseranas semipalmata* (AY309455)	
Apteryx australis	*Apteryx mantelli* (AY016010)	
Ardea herodias	*Ardea novaehollandiae* (NC_008551)	
Arenaria interpres	*Arenaria interpres* (NC_003712)	
Aythya americana	*Aythya americana* (NC_000877)	
Balaeniceps rex	*Balaeniceps rex* (GU071053)	
Bucorvus abyssinicus	*Bucorvus leadbeateri* (NC_015199)	
Buteo jamaicensis	*Buteo buteo* (AF380305)	
Cacatua sulphurea	*Cacatua moluccensis* (JF414239)	
Casuarius casuarius	*Casuarius casuarius* (AF338713)	
Cathartes aura	*Cathartes aura* (AY463690)	
Chalcopsitta cardinalis	*Melopsittacus undulatus* (NC_009134)	
Ciconia ciconia	*Ciconia ciconia* (NC_002197)	
Colibri coruscans	*Archilochus colubris* (NC_010094)	*Colibri coruscans* FGB (EU739462)
Columba livia	*Columba livia* (NC_013978)	*Geotrygon montana* TPM1 (EU737540)
Coracias caudata	*Eurystomus orientalis* (EU344978)	*Brachypteracias squamigera* CRYAA (EU737653), MUSK [exons only] (EU739772)
Corvus corone	*Corvus frugilegus* (NC_002069)	
Coturnix coturnix	*Coturnix japonica* (NC_003408)	
Crotophaga sulcirostris	*Crotophaga ani* (HM746794)	
Crypturellus soui	*Crypturellus tataupa* (AY016012)	
Cuculus canorus	*Eudynamys taitensis* (EU410487)	*Coccyzus americanus* RHO (EU737195), FBG [exons only] (EU739390), MYC (EU738272), MUSK [exons only] (EU739786)
Diomedea nigripes	*Thalassarche melanophris* (AY158677)	
Dromaius novaehollandiae	*Dromaius novaehollandiae* (NC_002784)	
Dryocopus pileatus	*Dryocopus pileatus* (NC_008546)	*Indicator maculatus* ALDOB (EU737855), RHO (EU737230)
Eudocimus albus	*Nipponia nippon* (NC_008132)	
Eudromia elegans	*Eudromia elegans* (NC_002772)	
Eudyptula minor	*Eudyptula minor* (AF362763)	
Eupodotis ruficrista	*Otis tarda* (NC_014046)	*Choriotis kori* [= *Ardeotis kori*] EEF2 (EU738599)
Falco mexicanus	*Falco peregrinus* (AF090338)	*Daptrius ater* MUSK [exons only] (EU739799)
Fregata magnificens	*Fregata* sp. (AP009192)	
Fringilla montifringilla	*Taeniopygia guttata* (DQ422742)	*Passer montanus* FGB (EU739142, EU739290, EU739455)
Gallus gallus	*Gallus gallus* (GU261713)	
Gavia immer	*Gavia stellata* (NC_007007)	
Geococcyx californianus	*Geococcyx californianus* (EU410488)	
Grus canadensis	*Grus grus* (NC_020577)	*Aramus guaranus* RHO (EU737173)
Haematopus ostralegus	*Haematopus ater* (AY074886)	
Larus marinus	*Larus dominicanus* (AY293619)	
Megalaima virens	*Pteroglossus azara* (NC_008549)	*Capito niger* TGFB2 [exons only] (EU737344), EEF2 [exons only] (EU738591)
Menura novaehollandiae	*Menura novaehollandiae* (NC_007883)	

Appendix 2. Continued.

Primary species for nuclear loci	Matching mt genome	Taxa sampled to improve sequence representation
Micrastur semitorquatus	*Micrastur gilvicollis* (NC_008548)	*Herpetotheres cachinnans* TGFB2 [exons only] (EU737391), EGR1 [exons only] (EU738961), FGB (EU739118, EU739266, EU739427), MUSK (EU739821)
Morus bassanus	*Morus serrator* (GU071056)	
Numida meleagris	*Numida meleagris* (NC_006382)	
Nyctibius grandis	*Nyctibius grandis* (EU344977)	*Nyctibius bracteatus* EEF2 (EU738651)
Oceanites oceanicus	*Pelagodroma marina* (KC875856)	
Pandion haliaetus	*Pandion haliaetus* (NC_008550)	
Pelecanus occidentalis	*Pelecanus conspicillatus* (DQ780883)	
Phaethon rubricauda	*Phaethon rubricauda* (NC_007979)	*Phaethon lepturus* CRYAA (EU737730), MUSK (EU739852)
Phalacrocorax carbo	*Phalacrocorax chalconotus* (NC_014679)	
Phoenicopterus chilensis	*Phoenicopterus roseus* (NC_010089)	
Podiceps auritus	*Tachybaptus novaehollandiae* (EF532936)	
Puffinus griseus	*Pterodroma brevirostris* (AY158678)	
Rallus limicola	*Porphyrio hochstetteri* (NC_010092)	*Sarothrura elegans* TPM1 (EU737596), *Himantornis haematopus* MUSK (EU739822)
Rhea americana	*Rhea americana* (AF090339)	
Rhynochetos jubatus	*Rhynochetos jubatus* (NC_010091)	*Eurypyga helias* EEF2 [introns/UTRs only] (EU738789), FGB (EU739107, EU739253, EU739413)
Smithornis rufolateralis	*Smithornis sharpei* (NC_000879)	*Pitta guajana* TPM1 (EU737582), EGR1 [introns/UTRs only] (EU738843)
Strix occidentalis	*Ninox novaeseelandiae* (NC_005932)	*Speotyto cunicularia* CRYAA (EU737757), FGB [exons only] (EU739490)
Struthio camelus	*Struthio camelus* (AF338715)	
Sylvia nana	*Sylvia crassirostris* (NC_010229)	
Tinamus guttatus	*Tinamus major* (NC_002781)	
Tockus camurus	*Aceros corrugatus* (HM755883)	
Trogon personatus	*Trogon viridus* (NC_011714)	
Tyrannus tyrannus	*Cnemotriccus fuscatus* (NC_007975)	*Mionectes macconnelli* MB (EU739991)
Tyto alba	*Tyto alba* (EU410491)	*Phodilus badius* RHO (EU737263)
Vidua chalybeata	*Vidua chalybeata* (AF090341)	

APPENDIX 3. Body mass estimates for internal nodes representing pairs of tip taxa, based on species data from the CRC Handbook of Avian Masses (Dunning, 2008). Species means were used, and when sequences were taken from multiple taxa (see Appendix 2), all sampled species were included in the average for that composite taxon. Node numbers correspond to those shown in Figure 2.

Node	Taxa included in comparison	Average body mass (g)
6. Casuariiformes	*Dromaius novaehollandiae*, *Casuarius casuarius*	391,000.0
8. *Crypturellus/Tinamus*	*Crypturellus soui*, *Crypturellus tataupa/Tinamus major*, *Tinamus guttatus*	534.7
13. *Gallus/Coturnix*	*Gallus gallus/Coturnix coturnix*, *Coturnix japonica*	467.3
16. *Anas/Aythya*	*Anas platyrhynchos*, *Anas falcata/Aythya americana*	1081.9
20. Mirandornithes	*Podiceps auritus*, *Tachybaptus novaehollandiae/Phoenicopterus chilensis*, *Phoenicopterus roseus*	1501.1
21. Columbiformes/Phaethontiformes	*Columba livia/Phaethon rubricauda*, *Phaethon lepturus*	507.1
25. Apodiformes	*Aerodramus vanikorensis*, *Apus apus/Colibri coruscans*, *Archilochus colubris*	14.6
31. *Geococcyx/Crotophaga*	*Geococcyx californianus/Crotophaga sulcirostris*, *Crotophaga ani*	238.3
32. Grues/Rallidae	*Grus canadensis*, *Grus grus*, *Aramus guaranus/Rallus limicola*, *Porphyrio hochstetteri*, *Sarothrura elegans*, *Himantornis haematopus*	1877.8
37. *Puffinus/Diomedea*	*Puffinus griseus*, *Pterodroma brevirostris/Diomedea nigripes*, *Thalassarche melanophris*	1967.2
41. Phalacrocoracoidea	*Phalacrocorax carbo*, *Phalacrocorax chalconotus/Anhinga anhinga*, *Anhinga rufa*	1429.1
44. Ardeidae/Threskiornithidae	*Ardea herodias*, *Ardea novaehollandiae/Eudocimus albus*, *Nipponia nippon*	1404.5
45. *Pelecanus/Balaeniceps*	*Pelecanus occidentalis*, *Pelecanus conspicillatus/Balaeniceps rex*	5018.7
48. *Arenaria/Larus*	*Arenaria interpres/Larus marinus*, *Larus dominicanus*	727.9
51. *Buteo/Pandion*	*Buteo jamaicensis*, *Buteo buteo/Pandion haliaetus*	1242.7
53. Strigiformes	*Strix occidentalis*, *Ninox novaeseelandiae*, *Speotyto cunicularia/Tyto alba*, *Phodilus badius*	323.3
57. Bucerotiformes	*Bucorvus abyssinicus*, *Bucorvus leadbeateri/Tockus camurus*, *Aceros corrugatus*	437.7
59. Pici	*Megalaima virens*, *Pteroglossus azara*, *Capito niger/Dryocopus pileatus*, *Indicator maculatus*	151.9
60. Alcedinidae/Coracioidea	*Alcedo leucogaster*, *Todiramphus sanctus*, *Momotus momota*, *Todus angustirostris/Coracias caudata*, *Eurystomus orientalis*, *Brachypteracias squamigera*	26.7
62. Falconidae	*Falco mexicanus*, *Falco peregrinus*, *Daptrius ater/Micrastur semitorquatus*, *Micrastur gilvicollis*, *Herpetotheres cachinnans*	496.3
64. Psittaciformes	*Chalcopsitta cardinalis*, *Melopsittacus undulatus/Cacatua sulphurea*, *Cacatua moluccensis*	312.0
67. Tyranni	*Smithornis rufolateralis*, *Smithornis sharpei*, *Pitta guajana/Tyrannus tyrannus*, *Mionectes macconnelli*	36.1
71. *Vidua/Fringilla*	*Vidua chalybeata/Fringilla montifringilla*, *Passer montanus*, *Taeniopygia guttata*	16.7

ASSESSING THE FOSSIL RECORD OF ASTERIDS IN THE CONTEXT OF OUR CURRENT PHYLOGENETIC FRAMEWORK[1]

Steven R. Manchester,[2] *Friðgeir Grímsson,*[3] *and Reinhard Zetter*[3]

ABSTRACT

The fossil record provides good evidence for the minimum ages of important events in the diversification and geographic spread of Asteridae, with earliest examples extending back to the Turonian stage of the Late Cretaceous (~89 million years ago [Ma]). Some of the fossil identifications accepted in previous considerations of asterid phylogeny do not stand up to careful scrutiny. Nevertheless, among major clades of asterids, there is good evidence for a range of useful anchor points. Here, we provide a synopsis of fossil occurrences that we consider reliable representatives of modern asterid families and genera. In addition, we provide new examples documented by fossil-dispersed pollen investigated by both LM and SEM studies including representatives of Loranthaceae, Amaranthaceae, Cornaceae (including *Nyssa* L., *Mastixia* Blume, *Diplopanax* Hand.-Mazz.), Sapotaceae, Ebenaceae, Ericaceae, Icacinaceae, Oleaceae, Asteraceae, Araliaceae, Adoxaceae, and Caprifoliaceae from Paleogene sites in Greenland, western North America, and central Europe, and of Lamiaceae and Asteraceae from the Middle to Late Miocene in northeastern China. We emphasize that dispersed pollen, taken along with megafossil and mesofossil data, continue to fill gaps in our knowledge of the paleobotanical record.

Key words: Angiosperms, Asteridae, Cenozoic, Cretaceous, paleobotany, palynology.

In the past few decades, our concept of what constitutes the Asteridae and the phylogenetic relationships among its constituents has evolved faster than the plants themselves. With the aid of abundant molecular sequence data, the relationships, both within and outside this major clade of angiosperms, have become increasingly well resolved (e.g., Soltis et al., 2011). The fossil record of Asterids is not as dense as that for other major groups, e.g., the Rosids, but there are many significant Cretaceous and Cenozoic fossils representing a range of Asterid clades.

The ages of Asterid clades have been inferred previously by fossil-calibrated molecular clock approaches (Bremer et al., 2004) and by direct inference from the position of the oldest fossils of component clades (Magallón et al., 1999; Crepet et al., 2004; Martínez-Millán, 2010). Martínez-Millán (2010) compared numerical estimates for the ages of Asterids and their major clades derived from molecular approaches with those derived directly from fossils and commented on the rather large discrepancies. The minimum age that molecular estimates inferred by modified molecular clock approaches calibrated with selected points from the fossil record generally exceeded by many millions of years (13–58.5 million years depending on the clade; table 1 in Martínez-Millán, 2010) those inferred by direct observation of available fossils in relation to currently held phylogenetic topologies. Based on available fossils, Martínez-Millán concluded that the Asteridae date back at least to the Turonian stage of the Late Cretaceous (89.3 million years ago [Ma]) and that its four main clades were already represented in the fossil record by the Late Santonian–Early Campanian (83.5 Ma). It remains debatable whether the substantial gap between the still older origination times of these clades as inferred from molecular estimates and their oldest physical evidence in the fossil record is due to analytical problems or failure to recover and recognize older fossils. Of course, the quality of these estimates depends in part on the reliability of fossils used in analyses. Suitable fossils need to be positioned with high confidence, both as to geologic age and systematic affinity.

In assessing the published fossil record of asterids and choosing which fossils to use from the literature

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for assessing the minimum age of specific asterid clades, Martínez-Millán (2010) applied a "filtering system" that led her to accept or reject published records based on completeness of the original author's presentation of the paleobotanical species. Three primary criteria were used to assess the acceptability of each fossil: (1) inclusion of the fossil in a phylogenetic analysis, (2) discussion of key characters that place the fossil in a group, and (3) list of key characters that place the fossil in the group. Reports that fulfilled one or more of these requirements were accepted as representing reliable records, while those that did not meet these standards were provisionally rejected (Martínez-Millán, 2010: 88). We agree in part with this method, yet it does not deal explicitly with the fossils and their characteristics but rather with the presentation style of the authors who identified those fossils.

Martínez-Millán (2010) tabulated fossil reports of various asterid clades, emphasizing the geologically earlier records for each clade, and indicating the organ represented, the age and geography of the fossil, and whether or not the report was accepted for calibrating nodes of the asterid phylogeny. These tables, including 261 records, are a helpful guide to relevant literature. Use of the strict filter criteria mentioned above resulted in some decisions with which we cannot agree, however. For example, *Marcgravia* L. and *Norantea* Aubl. pollen from the Middle Oligocene of Puerto Rico (Graham & Jarzen, 1969) and various reports of the paleoecologically important pollen records of the mangrove *Pelliciera* Planch. & Triana (e.g., Graham & Jarzen, 1969; Graham, 1977, 1999) were blanketly rejected. It is true that the authors did not include these fossils in a phylogenetic analysis and did not provide a thorough discussion of key characters that support identification to the indicated genera. The morphology of these grains (illustrated, e.g., in Graham, 1977) is sufficiently distinctive; however, the authors apparently did not deem it necessary to defend their assignment of the fossils that they documented by LM.

On the other hand, some of the records accepted in the same treatment by Martínez-Millán (2010) strain credulity. "Symmetrical, narrow ovate to elliptic shape, acute apex, entire and slightly undulated margin, brochidodromous venation, presence of intersecondary veins and percurrent tertiaries are the diagnostic features of the present fossil which are commonly seen in the modern leaves of *Diospyros* Linn. of the family Ebenaceae" (Prasad & Pradhan, 1998: 107). Presentation of this list of similarities resulted in an automatic decision of "acceptable" in Martínez-Millán's assessment; however, the single fragmentary specimen illustrated by the authors shows no characters unique to *Diospyros* L.; indeed, the above-quoted list of "diagnostic" characters for this entire-margined, pinnately veined leaf lacking its petiole and unknown in epidermal anatomy could apply equally well to numerous other genera in a wide array of angiosperm families not even confined to Asteridae.

The same can be said of the record of *Ardisia* Sw.: "the important morphological features of the present [fragmentary, entire-margined] fossil such as attenuate apex, entire margin, eucamptodromous venation, presence of intersecondary veins and the curvature of secondary veins near the margin strongly indicate its close affinity with the extant leaves of *Ardisia simplicifolia*" (Prasad & Pradhan, 1998: 106). Similarly, an entire-margined pinnately veined leaf lacking its petiole was accepted as Sapotaceae by Martínez-Millán (2010) based on the claim by Mehrotra (2000: 234): "The diagnostic characters of the fossil are: elliptic shape, rounded base, entire margin, eucamptodromous venation, moderate to wide acute angle of divergence of secondary veins, stout primary vein and presence of intersecondary veins. These features indicate the affinities of the fossil leaf with that of *Chrysophyllum* L. of Sapotaceae." We do not feel that these particular records are convincing because the identifications are based only on similarities, without indicating that these similarities are unique and/or whether they include any synapomorphies. Despite these disagreements over some of the records accepted and rejected, the timing and appearance of major asterid clades inferred by Martínez-Millán (2010) remain as reasonable estimates.

In their book reviewing the fossil record of all angiosperm orders, Friis et al. (2011) devoted a chapter to Asteridae—a review that had been conducted independently from that by Martínez-Millán (2010). Together, these references provide a good overview of our current understanding of the fossil record of Asterids. Here, we augment these reviews with additional informative examples and point out some prior misinterpretations. As a frame of reference, we use the phylogenetic topology provided by Soltis et al. (2011) in their study of angiosperm phylogeny based on 17 genes and 640 species. That phylogeny, abridged with emphasis on Asterids, is shown here in Figure 1. We consider examples from the fossil record in the sequence of Ericales, Cornales, Lamiids, and Campanulids. It would be desirable if fossil occurrences were preserved as whole plants with characters of all organs intact. This is never the case, however, so we rely on the

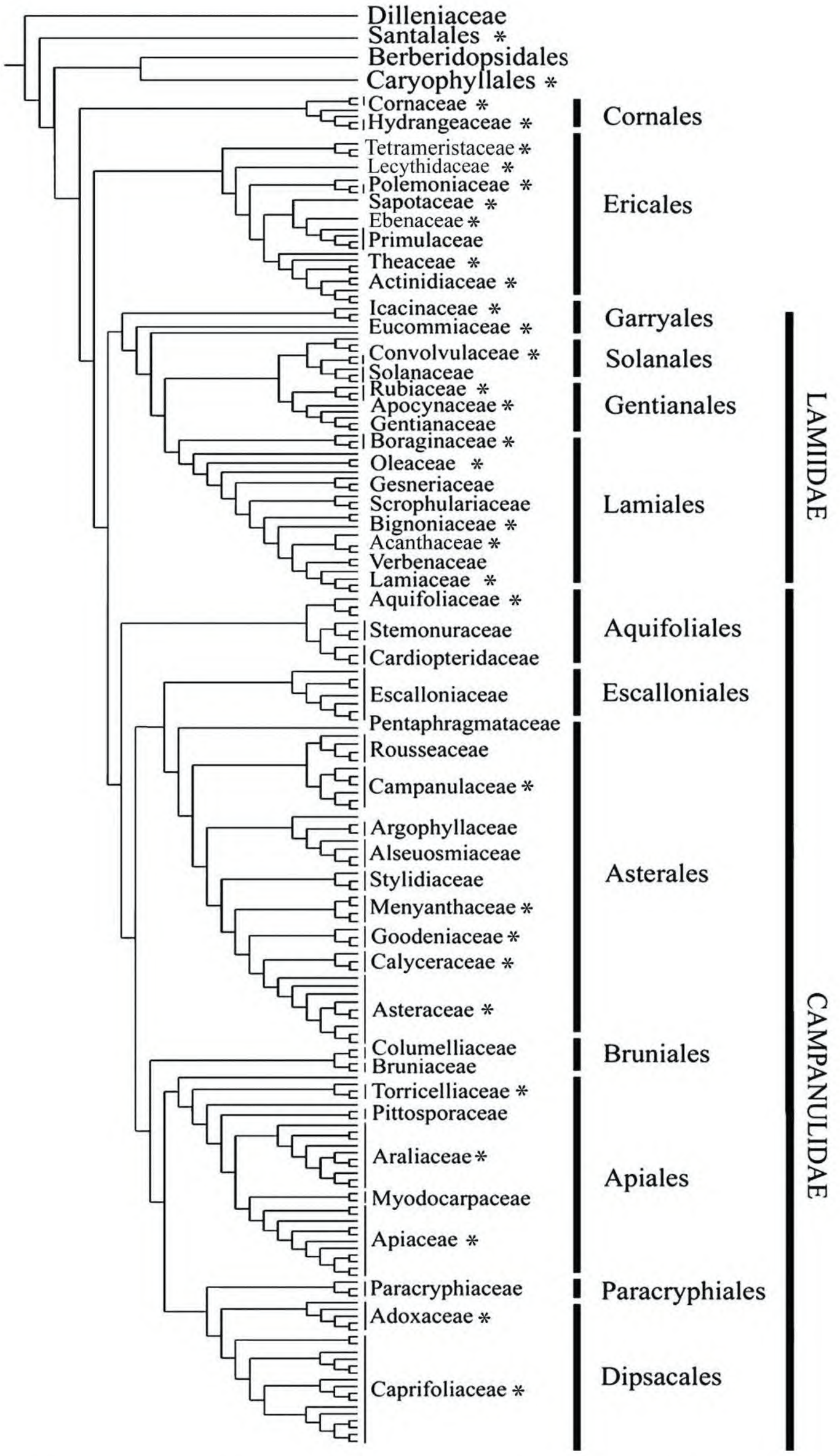


Figure 1. Phylogeny of asterids excerpted from the 17-gene analysis of angiosperms (Soltis et al., 2011). Asterisks indicate those families for which fossil remains are known.

disaggregated remains that happen to be preserved as fossils. In some instances, whole flowers are preserved in excellent detail, including the perianth, gynoecium with ovules and placentation intact, and stamens with in situ pollen preserved, yet we do not know the corresponding leaves or wood. In other cases we may have only leaves, only dispersed pollen, or just the wood. In some cases these individual organs or tissues are highly distinctive, with character combinations that appear to be unique to particular clades. The record of fossil flowers and fruits (Friis et al., 2011) is particularly important, especially for recognizing some of the earliest members of the Asterids. The dispersed pollen record is also very useful and deserves more attention than it has received in previous reviews. In this article, we review fossil examples, including pollen, flowers, leaves, wood, fruits, and seeds, and we show how they are spread across the phylogeny as we currently understand it. Most are gleaned from the literature, but we also introduce new examples, especially of dispersed fossil pollen.

The identifications of fossil pollen grains to modern genera commonly found in the literature are often based exclusively on transmitted LM, without the resolution of informative fine details that can be provided by SEM. In some instances, the morphological characters resolvable by LM are sufficient to provide confident identification, e.g., *Alnus* Mill., *Carya* Nutt., *Fagus* L., *Liquidambar* L., *Quercus* L., *Ulmus* L., and other highly distinctive grains. But in many cases, pollen grains of unrelated taxa can be nearly indistinguishable unless SEM details are available. In the present work, we rely on the combined information provided by both LM and SEM of the same grains.

Materials and Methods

In addition to citing from the literature in this review, we include new documentation of fossil pollen grains from selected Late Cretaceous, Paleocene, Eocene, and Miocene localities in the Northern Hemisphere. Sedimentary rock samples were processed and pollen grains were isolated from the residue using standard techniques (e.g., Grímsson et al., 2008, 2011), and individual grains were removed and manipulated for imaging first by LM and then by SEM, using the single grain technique (e.g., Zetter, 1989; Ferguson et al., 2007). This method documents more characters than would be available from LM or SEM alone, and it ensures that the observed morphological features are obviously from the same species. For investigating the likely affinities of these pollen relative to extant taxa, it has been important to compare them with as many modern genera as possible. Broad systematic treatments of the palynology of particular regions (e.g., Li et al., 2009; Miyoshi et al., 2011) as well as the specialty literature on pollen of particular families or genera, cited later in this article, have been very useful.

There are eight sources for the fossil pollen grains presented herein by combined SEM and LM: the Cretaceous (Early Campanian) of Elk Basin, Wyoming, U.S.A.; the Early Paleocene of Agatdalen, western Greenland; the Late Paleocene of Almont, North Dakota, U.S.A.; the Middle Eocene of Hareøen, western Greenland; Princeton, British Columbia, Canada; and Stolzenbach, Germany; the Late Eocene of Profen, Germany; and the Miocene of Heilongjiang Province, China. Geographic and geologic source information for these sites is provided below.

Elk Basin, Wyoming (Early Campanian)

The sedimentary samples originate from the Eagle Formation of Elk Basin, a valley bordering the Wyoming and Montana State line. Elk Basin comprises several outcrops with both marine and terrestrial, Upper Cretaceous to Lower Cenozoic, sedimentary sequences. The Eagle Formation is locally divided into two units: the Lower Virgelle Sandstone Member and the Upper Eagle Beds. The upper beds are composed of alternating sandstones, carbonaceous mudstones, siltstones, shales, clays, and lignites (Hicks, 1993; Van Boskirk, 1998). All of the plant macrofossils described by Van Boskirk (1998) and the sediment samples used for this palynological study originate from the upper part of the Upper Eagle Beds, from below the 1-m-thick bentonite layer positioned ca. 10 m below the top of the formation. The Eagle Formation is succeeded by a regionally extensive ashfall bed named the Ardmore Bentonite, which comprises the basal unit of the Claggett Shale Formation (cf. Hicks, 1993). Biostratigraphic and magnetostratigraphic studies of the Elk Basin by Hicks (1993) suggest that the lowest part of the Upper Eagle Beds coincides with the *Scaphites hippocrepis* III ammonite zone, and the ca. upper two thirds (composing the fossilized plant material) of the beds lie within the *Baculites* sp. (smooth; early) zone. According to Obradovich (1993) and Gradstein et al. (1995), the base of the *Baculites* sp. (smooth; early) zone started at ca. 81 Ma and continued for a period of ca. 0.5 million years. A similar, but slightly older, age for the plant fossiliferous sediments was obtained by isotopic data from the previously mentioned bentonite layer positioned close to the top of the Eagle Formation, dated to 81.13 ± 0.5 Ma (Hicks, 1993). This suggests that the plant-bearing unit of the Eagle Formation is Early Campanian in age and approximately 82–81 Ma.

Agatdalen, Greenland (Early Paleocene)

The fossil pollen from this locality originate from phosphoritic nodules of Agatdalen Valley in the central part of the Nuussuaq Peninsula, western Greenland. Early Paleocene sedimentary rocks in the northern part of the valley have been partly assigned to the Agatdal Formation (Koch, 1963; Dam et al., 2009). The type locality/section of the Agatdal Formation is the "Store Profil" or "Big Section" in Turritellakløft Gorge (see Dam et al., 2009). The phosphoritic nodules containing the fossil pollen were collected between the years 1948 and 1964 at the Store Profil type section from sediments of the Agatdal Formation. The stratigraphic position of the Agatdal Formation (Koch, 1963; Dam et al., 2009), its exceptionally rich fossil marine fauna (e.g., Bendix-Almgreen, 1969; Hansen, 1970; Rosenkrantz, 1970; Szczechura, 1971; Floris, 1972; Perch-Nielsen, 1973; Hansen, 1976;

Kollmann & Peel, 1983; Collins & Wienberg Rasmussen, 1992; Petersen & Vedelsby, 2000), as well as correlations with radiometric dates of overlying formations (see Storey et al., 1998; Dam et al., 2009) show that the Agatdal Formation is Upper Danian, between 64 and 62 Ma.

ALMONT, NORTH DAKOTA (LATE PALEOCENE)

Silicified shales of the Sentinel Butte Formation, exposed about 9.5 km northwest of New Salem, North Dakota, have yielded well-preserved leaves, fruits, seeds, flowers (Crane et al., 1990), and dispersed pollen (Zetter et al., 2011). The lacustrine deposit is considered to be Late Paleocene (Tiffanian 3) based on pollen and regional stratigraphic correlations (Kihm & Hartman, 1991).

PRINCETON CHERT, BRITISH COLUMBIA (MIDDLE EOCENE, EARLY TO MIDDLE LUTETIAN)

This outcrop is located along the east bank of the Similkameen River, ca. 8.4 km south of the town of Princeton, British Columbia, Canada. The silicified and fossil-rich sedimentary rocks that compose the Princeton Chert beds belong to the uppermost part of the Allenby Formation, which is part of the Princeton Basin. The basin is a northerly trending trough comprising various volcanic and sedimentary rock units of Eocene age that form the Princeton Group. Jurassic and Triassic rocks make up most of the basement and outline/margins for the basin (McMechan, 1983; Read, 1987, 2000). The Princeton Group is divided into two formations: the Lower Cedar Formation is mostly of volcanic origin and the Upper Allenby Formation is constructed of various sedimentary rock units. The lowest two major units of the Allenby Formation are the Sunday Creek Conglomerate and the Hardwick Sandstone. These units are overlain by the Vermilion Bluffs Shale, the Summer Creek Sandstone, and the Ashnola Shale (McMechan, 1983; Read, 1987, 2000). The Ashnola Shale includes in its uppermost part the Princeton Chert beds (Read, 2000; Mustoe, 2011). The samples used for this study originate from chert bed 43, from the uppermost quarter of the Princeton Chert sequence. The exact age of the Princeton Chert beds is difficult to pinpoint, but studies based on fossil mammals, fish, and plants (e.g., Russell, 1935; Gazin, 1953; Rouse & Srivastava, 1970; Wilson, 1977, 1982; Cevallos-Ferriz et al., 1991; Pigg & Stockey, 1996) suggest an Eocene age. Radiometric dating indicates that most of the volcanic rocks of the Cedar Formation and the sedimentary rocks of the lower to middle part of the Allenby Formation are 53–48 Ma, and are therefore Early Eocene in age. The uppermost part of the Allenby Formation is dated to ca. 46 Ma, suggesting that the Princeton Chert beds are of Middle Eocene age (Early to Middle Lutetian).

HAREØEN, GREENLAND (MIDDLE EOCENE, LATE LUTETIAN TO EARLY BARTONIAN)

Fossil pollen from Hareøen originate from sediments of the Middle Eocene Aamaruutissaa Member of the Hareøen Formation, on the island of Hareøen, off the western shore of Greenland (Grímsson et al., 2014, 2015). Plant macrofossils from Hareøen described by Heer (1868–1883) and Nathorst (1885) originate from the same member. The fossil pollen derived from a resinite-rich coal bed in the intrabasaltic Aamaruutissaa Member, which forms the lower part of the Hareøen Formation (Hald, 1976, 1977). The Aamaruutissaa Member overlies the Early Eocene Kanissut Member. The Kanissut Member belongs to the Naqerloq Formation, which is dated to 56–54 Ma (Storey et al., 1998; Dam et al., 2009). The Aamaruutissaa Member is overlain by the Late Eocene Talerua Member (Hald, 1976, 1977). The Talerua Member lava flows have been dated radiometrically to 38.8 ± 0.5 Ma (Schmidt et al., 2005), suggesting that the underlying plant fossil–bearing sediments of the Aamaruutissaa Member are slightly older. Pollen analyses suggest a Late Lutetian to Early Bartonian (42–40 Ma) age for the plant-bearing sediments (Grímsson et al., 2014, 2015).

PROFEN, SACHSEN, GERMANY (MIDDLE EOCENE)

The Profen locality is an open-cast coal mine in Sachsen-Anhalt State in central Germany. The sediments are part of the Weißelster Basin that composes Middle Eocene to Pliocene strata. The geology of the Profen locality has been outlined by Pälchen and Walter (2011), indicating that the oldest part of the Profen sediments, from which our palynological samples originate, are of Middle Eocene (Bartonian) age. The palynoflora from the Eocene sediments of Profen was described using LM by Krutzsch and Lenk (1973). The palynoflora is clearly of Eocene age, with most palynomorphs indicative of Middle Eocene but also with some elements normally suggesting an early Late Eocene age (Krutzsch & Lenk, 1973; R. Zetter, pers. obs.).

STOLZENBACH, HESSEN, GERMANY (MIDDLE EOCENE)

The Stolzenbach locality is an underground coal mine positioned just south of Kassel in the Borkener brown coal area. The geological settings and the plant macrofossil content of the sedimentary succession

within the coal mine were originally described by Oschkinis and Gregor (1992). The Eocene sediments have yielded numerous plant macrofossils, fossil insects, and vertebrate remains as well as an extremely rich palynoflora (e.g., Tobien, 1961; Hottenrott et al., 2010; Gregor & Oschkinis, 2013; Gregor et al., 2013). The palynological samples were taken from thin clay units within the lignites of the underground coal mine (Hottenrott et al., 2010). The vertebrate fauna described from the succession (Tobien, 1961; Gregor et al., 2013) and the composition of the microflora (R. Zetter, pers. obs.) suggest a Middle Eocene age for the sediments containing the fossils.

BEIPAIZI, NORTHEAST CHINA (MIDDLE TO LATE MIOCENE)

This pollen sample was collected from the Daotaiqiao Formation located at Beipaizi, about 4 km north of Sifangtai Village, Huanan County in Heilongjiang Province, northeast China (Grímsson et al., 2012). The geological settings and sedimentary succession from where the sample was collected have been described in detail by Liu et al. (1995, 1996) and Leng (1997, 2000a, 2000b). The sample originates from finely laminated, dark gray claystone, which is rich in fossil plants and skeletons of freshwater fish and insects. The claystone belongs to the lower part of a sedimentary succession composing the Daotaiqiao Formation. The age of the Daotaiqiao Formation has been assigned to the late Middle Miocene to early Late Miocene (approximately 12–11 Ma) by correlating well-dated fossil macrofloras and microfloras in East Asia (Liu et al., 1995, 1996; Leng, 1997, 2000a, 2000b; Liu, 1998) and by the correlation of vertebrate faunas, including various fossil freshwater fish (Chang et al., 1996) and fossils of terrestrial mammals (Qi, 1992).

Results

LOWER ASTERIDAE

The Dilleniales are poorly documented in the fossil record. We are not aware of any substantiated records of this order. Leaves attributed to *Tetracera* L. and *Dillenia* L. from the Late Eocene of Oregon (Chaney & Sanborn, 1933) do resemble leaves of some extant species of those genera, but they are also difficult to distinguish from those of Fagaceae and Ticodendraceae.

SANTALALES

This order is relatively well represented in the fossil record, based on pollen of Loranthaceae, with examples of extant genera recognizable back to the Early Eocene in the Southern Hemisphere (Macphail et al., 2012) and Middle Eocene of the Northern Hemisphere (Zetter et al., 2014). Loranthaceae have distinctive triangular oblate pollen grains that are typically concave sided and syncolpate, with distinctive variation in ornamentation and aperture configuration among genera (e.g., Feuer & Kuijt, 1980, 1985). Combined LM and SEM study of dispersed fossil grains indicates that the family was widespread by the Middle Eocene, with examples conforming to modern genera known from the Middle Eocene of western Greenland (Fig. 2A–C) and central Europe (Fig. 2D–F).

CARYOPHYLLALES

This order is represented mainly by fossils in the Amaranthaceae and Polygonaceae. The Amaranthaceae lack much of a megafossil record but are well represented by dispersed fossil pollen. Pollen of Amaranthaceae (including Chenopodiaceae) are distinctive, nearly spheroidal, pantoporate grains with numerous annulate pores. Although appearing nearly psilate by LM, SEM reveals echinate to microechinate and perforate sculpturing (Nowicke, 1994). Fossil grains of this kind, commonly referred to the genus *Chenopodipollis* Krutzsch, among other names, are known from the Maastrichtian Hell Creek Formation (Nichols, 2002), the Paleocene of North Dakota (Fig. 3) (Zetter et al., 2011), and the Late Eocene of Colorado (Bouchal, 2013) and are common in the fossil record through at least the Miocene (reviewed, e.g., by Muller, 1981), but the assignment to individual extant genera is not possible because they overlap in morphology. *Caryophylloflora paleogenica* G. J. Jord. & Macphail, an inflorescence containing periporate pollen in situ, was recovered from the Middle to Late Eocene of northeastern Tasmania (Jordan & Macphail, 2003). In general, however, the megafossil record of Caryophyllales remains poorly documented.

Winged fruits of Polygonaceae have been identified from the Late Cretaceous (Maastrichtian: *Polygonocarpum johnsonii* Manch. & O'Leary) and Late Paleocene (*Polygonocarpum curtisii* Manch. & O'Leary, *Podopteris antiqua* Manch. & O'Leary) of North Dakota (Manchester & O'Leary, 2010). McIver and Basinger (1993) also identified as Polygonaceae the distinctive leaves of *Paranymphaea crassifolia* (Newberry) Berry, common in the Lower Paleocene of North America.

Friis et al. (2011) consider the pollen resembling *Bougainvillea* Comm. ex Juss. of the Nyctaginaceae from the Late Campanian of Sakhalin, Russia (Takahashi, 1997), to be a significant early record for

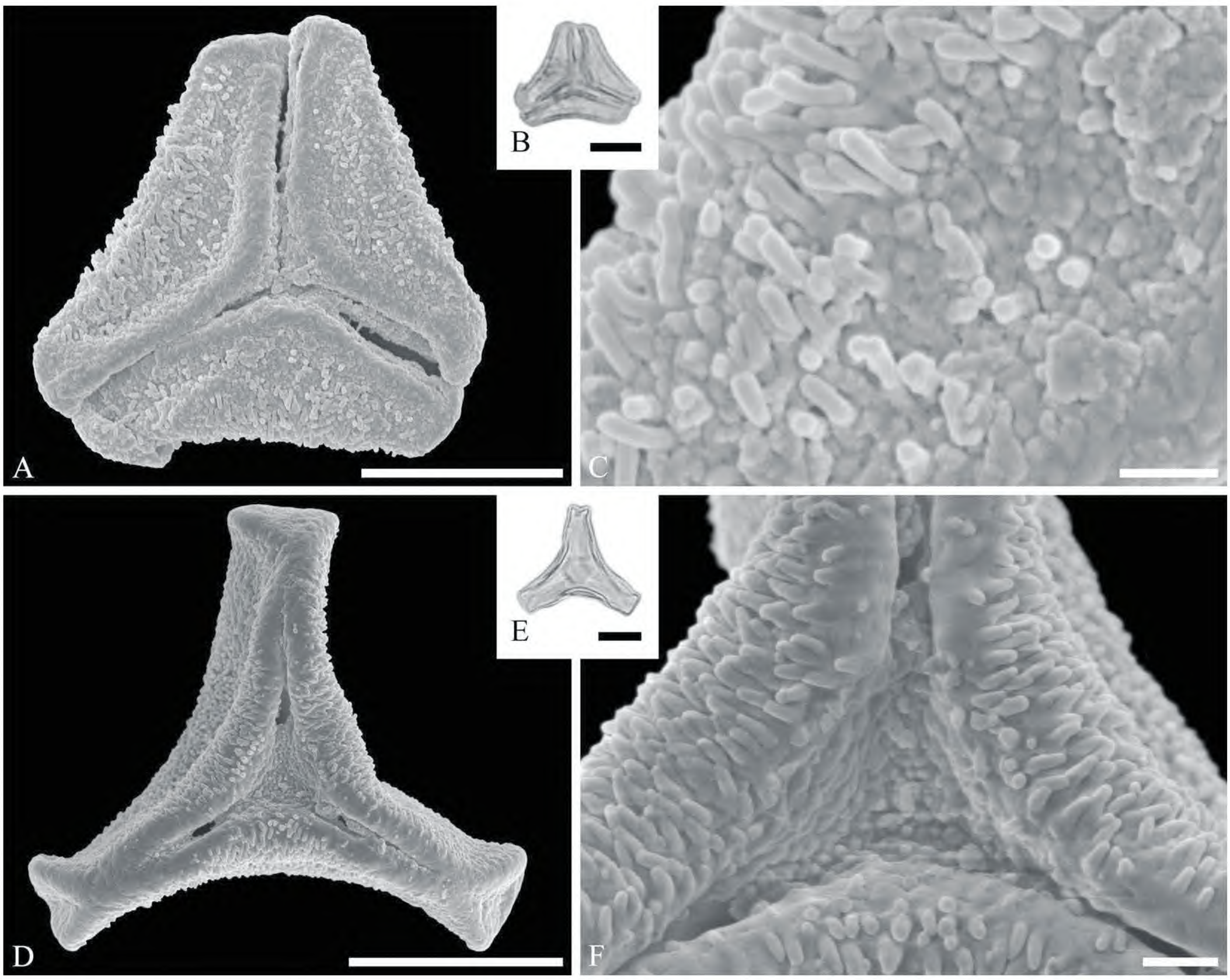


Figure 2. Pollen of Loranthaceae from the Middle Eocene of Greenland and Germany. A–C. Hareøen, western Greenland, gen. et spec. indet. showing prominent triradiate syncolpus. —A. SEM, polar view. —B. LM of same grain, polar view. —C. Close-up showing verrucate and bacculate ornamentation in area of mesocolpium. D–F. Profen, central Germany, gen. et spec. indet. —D. SEM, polar view. —E. LM, polar view. —F. Close-up showing sculpturing elements in polar area and mesocolpium. Scale bars: A, B, D, E = 10 μm; C, F = 1 μm.

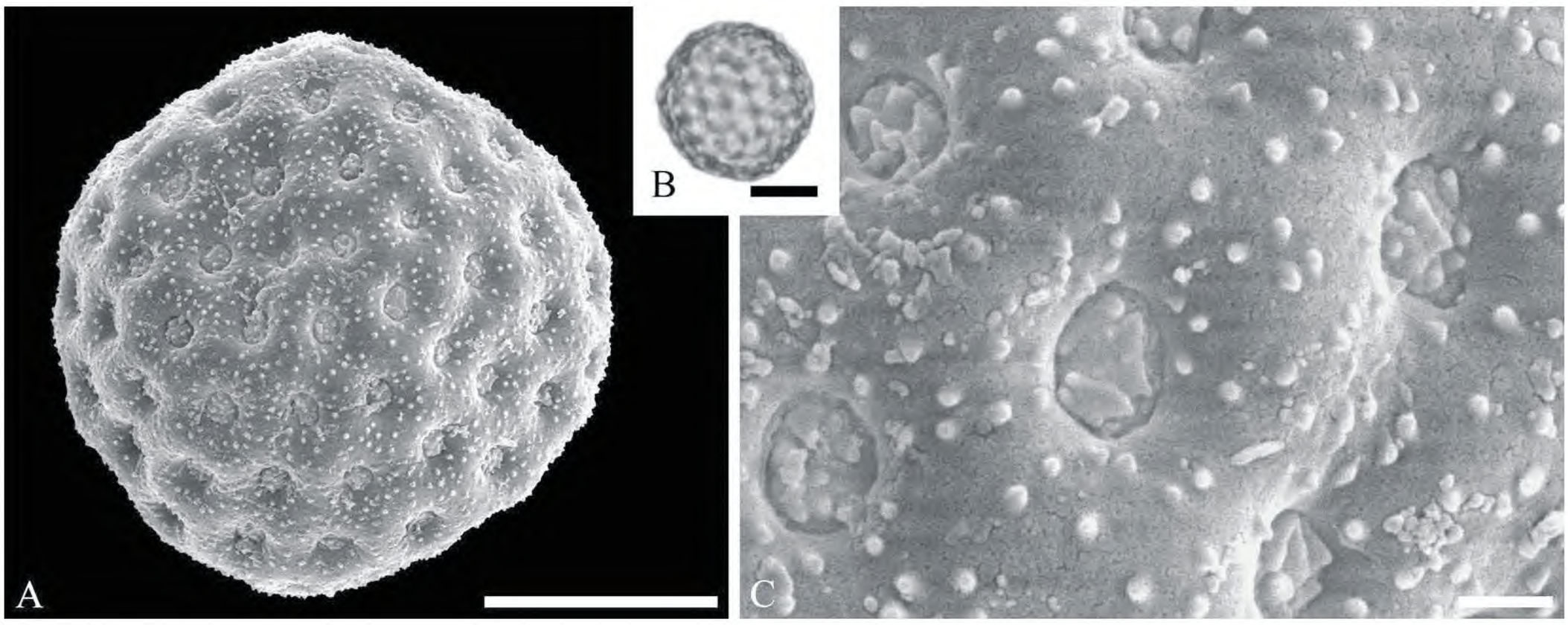


Figure 3. Pollen of Amaranthaceae from the Late Paleocene of Almont, North Dakota, U.S.A. A–C. Amaranthaceae gen. et spec. indet. —A. SEM of globose, multiporate grain. —B. LM of same grain. —C. Close-up showing surface sculpture of pollen and apertures with membrane. Scale bars: A, B = 10 μm; C = 1 μm.

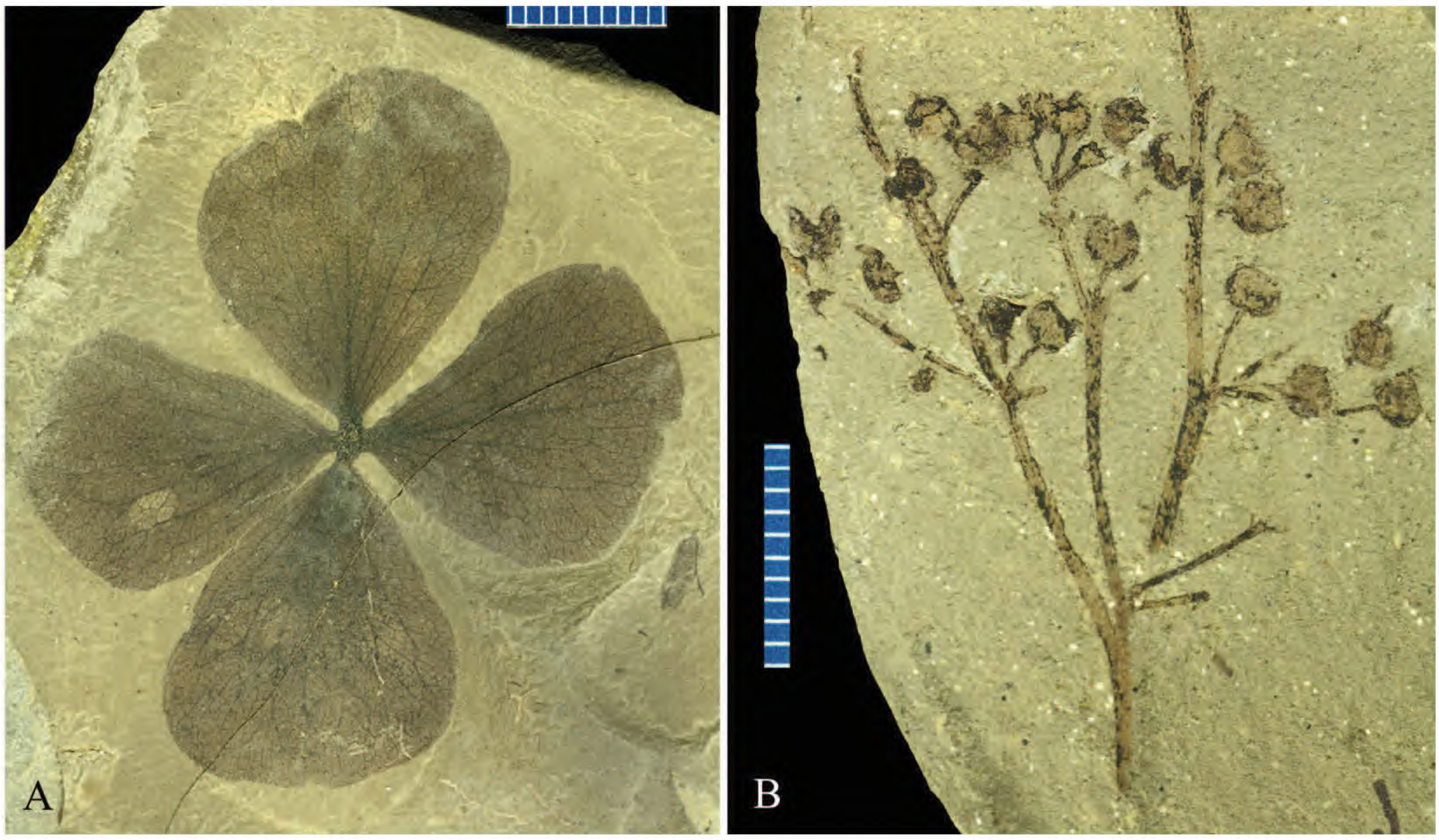


Figure 4. *Hydrangea knowltonii* flower and fruiting remains from the Middle Eocene Clarno Formation of West Branch Creek, Oregon, U.S.A. —A. Sterile showy perianth (UF-230-19187). —B. Paniculate infructescence showing fruits with persistent apically divergent styles (UF-230-18155). Scale bars = 1 cm.

this clade. Pollen of Nyctaginaceae is also reported with SEM as well as LM from the "Eocene" of Argentina (Zetter et al., 1999), but the correct age (?Eocene to ?Miocene) of these sediments has been questioned (e.g., Zamaloa, 2000; García-Massini et al., 2004). The tricolpate grains are distinctive by their aperture membranes consisting of masses of partly fused angular microechini and having a nexine that is much thinner than the sexine and a tectum with spaced microechini (pl. 3, figs. 10–12 in Zetter et al., 1999).

CORNALES

The Cornales are well represented in the fossil record, with Hydrangeaceae, Cornaceae (including *Nyssa* L. and *Mastixia* Blume), and Davidiaceae all extending back at least to the Campanian (~72 Ma) based on well-preserved, character-rich fruits. The oldest example of Hydrangeaceae is the charcoalified flower and fruit known as *Tylerianthus crossmanensis* Gandolfo, Nixon & Crepet 1998 from the Turonian of New Jersey. The extant genus *Hydrangea* L. is readily recognized in the fossil record based on compressed showy sterile flowers and fruits from the Early to Middle Eocene (Fig. 4A, B) (Manchester, 1994; Mustoe, 2002) and Oligocene (Meyer & Manchester, 1997). Permineralized Eocene *Hydrangea* fruits from Oregon were shown to contain winged seeds, a feature found in this genus today only in the Asian species *H. anomala* D. Don (Manchester, 1994).

Cornus L. is recognized by its distinctive leaves with eucamptodromous secondary veins and calcified T-shaped trichomes. Leaves showing these diagnostic characters have been confirmed from the Paleocene of Russia and western North America (Manchester et al., 2009b). Fruits of *Cornus* are also readily recognizable by a combination of characters including epigyny, two or more locules with dorsal germination valves, endocarp composed mostly of isodiametric cells, and transeptal placental bundles (Eyde, 1988). Fruits with endocarp wall cavities, distinctive of *Cornus* subg. *Cornus*, have been described in detail from the Paleocene of North America and Early Eocene of England (Manchester et al., 2010). Dispersed pollen of *Cornus* is also distinctive, being echinate and tricolporate with H-shaped endoapertures (thinning of the endexine with lamellation) (Ferguson, 1977, 1978). Such pollen is confirmed based on SEM studies from Paleocene (Zetter et al., 2011) and younger strata and has the potential to be traced back to the Cretaceous.

The oldest evidence for Nyssoideae is *Hironoia fusiformis* Takahashi, Crane & Manchester 2002, a fruit from the Early Coniacian of Japan. The fruits were thick-walled and composed mainly of fibers, with three or four locules, each containing one pendulous seed and opening by a single dorsal valve

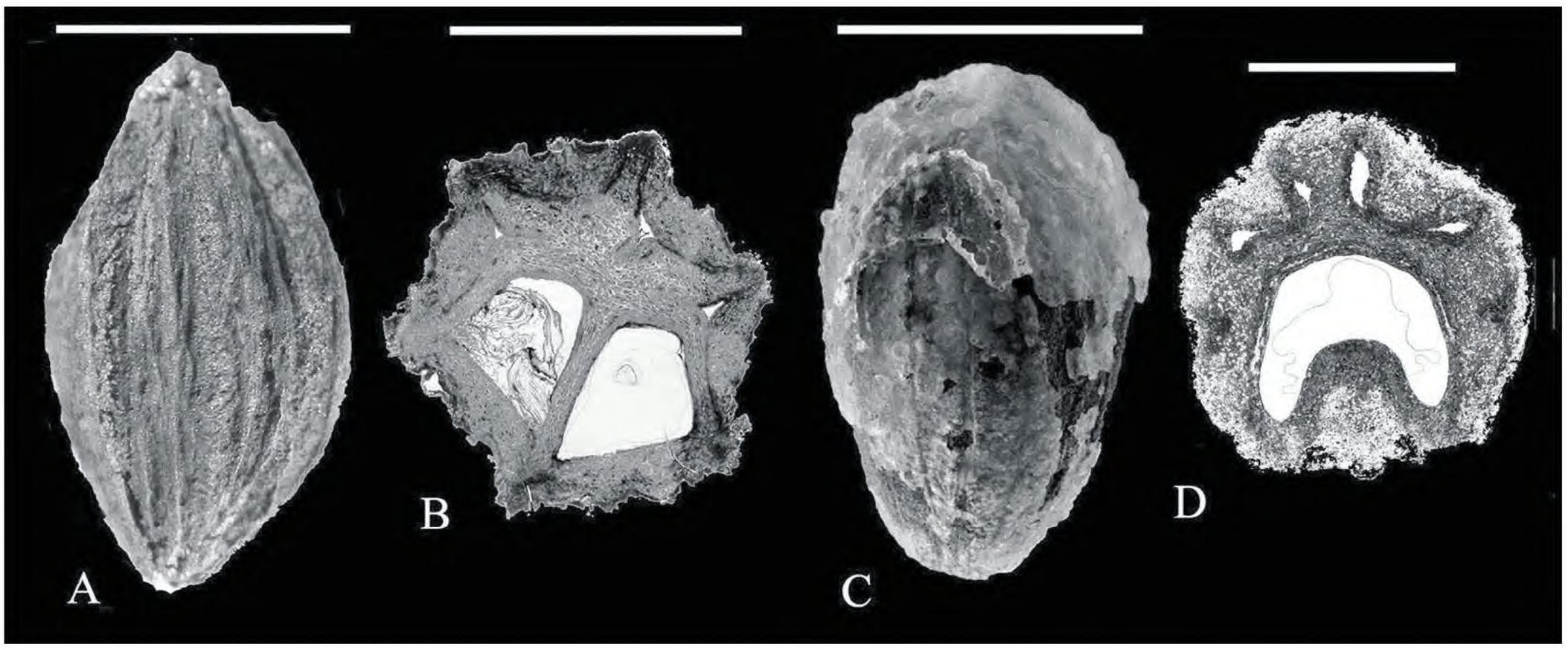


Figure 5. Cornalean fruits from the Late Cretaceous (Late Campanian) of Drumheller, Alberta, Canada. —A, B. *Davidia* Baill. sp. —C, D. *Nyssa* L. sp., Tyrrell Museum. Scale bars: A, C = 5 mm; B, D = 3 mm.

fruit, and preserved persistent epigynous tepals and a single narrow style. Absence of an axial vascular strand is a distinctive feature that it shares with most extant Cornaceae. The presence of fibers, rather than isodiametric sclereids, is a feature distinguishing this from *Cornus* and indicating similarity with Nyssoideae and Mastixioideae (Takahashi et al., 2002).

Subsequent records of Cornales include *Davidia* Baill. from the Late Campanian of Alberta, Canada, based on permineralized fruits (Fig. 5A, B) (Serbet et al., 2004). These early fruits of *Davidia* conform to the modern genus in having multiple, radially arranged, single-seeded locules, fibrous endocarp construction, and elongate dorsal germination valves. They closely resemble the modern genus morphologically and anatomically, but differ from the modern species of *Davidia* by their smaller size and fewer locules (five to six vs. six to nine). Other features of the plant, e.g., the leaves and inflorescence structure, remain unknown, so it is not proven whether this represents the stem or crown of *Davidia*. By the Paleocene, however, *Davidia* is clearly recognizable on the basis of well-preserved infructescences, showing the scars of showy bracts, plus fruits and foliage fitting well to the modern genus. Only the smaller size of the fruit and the lack of a median rib over each locule distinguish the Paleocene species *D. antiqua* (Newberry) Manchester from the modern *D. involucrata* Baill. (Manchester, 2002). Although *Davidia* is endemic to China today, it was present in the Paleocene of Asia and in the Paleocene to Late Eocene (Manchester & McIntosh, 2007) of western North America.

Fruits conforming morphologically and anatomically to *Nyssa* have also been identified from the Campanian of Alberta, Canada, the same locality yielding the oldest *Davidia* fruits mentioned above. They are trilocular with single-seeded locules that are C-shaped in cross-section, having dorsal, apically positioned germination valves (Fig. 5C, D). Although not formally named yet (Serbet & Manchester, in prep.), the fruits conform to a kind that is common in the Paleogene, formerly called *Palaeonyssa* Reid & Chandler. Eyde (1997: 105) noted that the distinction between *Palaeonyssa*, having three or four locules, from *Nyssa* "is probably not justified because plurilocular fruits also occur in extant species of *Nyssa*, particularly in *N. talamancana* of Costa Rica and Panama."

Extinct members of Nyssoideae include a widespread North American Paleocene plant, known by its infructescences and fruits as *Amersinia* Manch., Crane & Golovn. This plant bore obovoid, trilocular (occasionally tetralocular) fruits in ellipsoidal heads. The fruits have a persistent, epigynous disk and lack a central vascular strand (strands are scattered through the septa, as in *Davidia*). Each locule bore a single seed and had a dorsal germination valve in the apical half. The associated leaves, *Beringiaphyllum cupanioides* (Newberry) Manch., Crane & Golovn., had long petioles and laminae somewhat similar to those of *Davidia*, but with teeth more rounded and usually confined to the upper portion of the lamina. The small number of locules (three and rarely four), however, is more similar to that of *Camptotheca* Decne. and *Nyssa*. The epigynous disk of *Amersinia* relates it to other modern Cornales, except *Davidia*, which in modern species (and apparently in fossils) lacks this feature. *Amersinia* and *Beringiaphyllum* are found in the Paleocene of eastern Asia as well as North America (Manchester et al., 1999). *Browniea serrata* (Newberry) Manch. & Hickey represents another extinct genus of the

Paleocene, in western North America, that was apparently closely related to modern *Camptotheca*. It is known from associated infructescences, fruits, flowers with pollen in situ, and leaves (Manchester & Hickey, 2007).

Curtisia Aiton, the sole member of Curtisiaceae, also belongs to the Cornales and resembles Cornaceae in having woody fruits with single-seeded locules and dorsal germination valves, but is distinct in having a central vascular bundle between its four locules (Eyde, 1988). Although it lives today in southern Africa, the genus is known based on well-preserved fruits from the Early Eocene London Clay flora (Manchester et al., 2007).

Noferinia fusicarpa Lupia, Herendeen & Keller, from the Santonian of Georgia, U.S.A., bore dense headlike infructescences of numerous fusiform fruits, each with an epigynous perianth, and three to rarely four connate carpels with recurved style arms (Lupia et al., 2002). The fruits lack an axial bundle—a feature consistent with Cornaceae—but the presence or absence of germination valves was not documented. Pollen from the stamens is similar to that of *Nyssa* but with a more coarsely reticulate tectum.

Although Mastixioideae contain only two genera today, *Mastixia* (now distributed from East Asia to Malesia) and *Diplopanax* Hand.-Mazz. (Southwest China and Vietnam), this subfamily was diverse and common in western North America and Europe based on fossil fruits. Knobloch and Mai (1986) recognized five species of extinct mastixioids from the Late Cretaceous (Maastrichtian) of Walbeck and Eisleben, Germany. These show full-length dorsal germination valves, one of the diagnostic features of Mastixioideae, distinct from Nyssoideae (which has its germination valves confined to the apical half of the fruit). Detailed anatomical data are not yet documented for these species, but they do appear, on the basis of available characters, to be appropriately assigned to Mastixioideae. Mastixioid fruits are also known in the Paleocene of North Dakota (Manchester & Pigg, in prep.) and in the Eocene of western North America (Tiffney & Haggard, 1996; Stockey et al., 1998), England, and Germany (Mai, 1993). These include the two modern genera as well as extinct genera with novel combinations of fruit characters. Dispersed pollen of Mastixioideae are recognizable by the combination of H-shaped thinnings of endoaperture, shared with *Cornus*, in combination with a non-echinate, microverrucate, and perforate tectum. Examples are illustrated here from the Early Paleocene of Agatdalen, in western Greenland (Fig. 6A–C), and the Middle Eocene of Stolzenbach, Germany (Fig. 6D–F).

Alangium Lam. is well recognizable on the basis of fossil fruits (Eyde et al., 1969; Manchester, 1994) and wood (Scott & Wheeler, 1982) back to the Eocene. The oldest examples are fruits from the Early Eocene London Clay (Reid & Chandler, 1933). Fossil pollen of *Alangium* also has been widely recognized (Morley, 1982; Krutzsch, 1989; Martin et al., 1996), and some kinds of extant *Alangium* pollen are truly distinctive morphologically (Eyde et al., 1969). However, part of the morphological range of *Alangium* pollen overlaps with that of *Pelliciera* (Tetrameristaceae), presenting problems for distinguishing some of the fossil grains (discussed next section).

ERICALES

Among Ericales, the Actinidiaceae, Ericaceae, Polemoniaceae, Sapotaceae, Styracaceae, Symplocaceae, and Theaceae were well established by the Middle Eocene (~47 Ma) based on fossil reproductive structures, including fruits, seeds, and/or pollen. Flowers of several extinct genera, e.g., *Palaeoenkianthus* Nixon & Crepet 1993 and *Raritaniflora* Crepet, Nixon & Daghlian 2013 from New Jersey, extend this clade back to Turonian (~92 Ma). We can add some significant records to the extensive listing of ericalean fruits, seeds, wood, etc., in table 4 of Martínez-Millán (2010).

The Marcgraviaceae together with Tetrameristaceae and Balsaminaceae are currently seen to form a clade that is as sister to the rest of the Ericales (Soltis et al., 2011). Fossil records for *Marcgravia* have been reported based on pollen but by LM alone, and it is not clear that their morphology is unique to the family. In the Tetrameristaceae (currently circumscribed to include Pellicieraceae), the mangrove genus *Pelliciera* has been identified in the Neotropical fossil record from the Middle Eocene onward (Graham, 1977), based on rather distinctive dispersed pollen grains named *Lanagiopollis crassa* (Van der Hammen & Wymstra) Frederiksen. Frederiksen (1988) recognized *L. crassa* from the Middle Eocene Tallahatta Formation of the southeastern United States, and considered it likely to be *Pelliciera*, but commented on the difficulty of distinguishing pollen of this genus from that of unrelated extant genus *Alangium*: "Although fossil pollen of *Alangium* and *Pelliceria* can probably be distinguished, it does not seem worthwhile to have separate form-genera for the two types" (Frederiksen, 1988: 56). It would be desirable to investigate these grains by SEM as well as LM, but most records were deemed acceptable by Muller (1981) and Graham (1977).

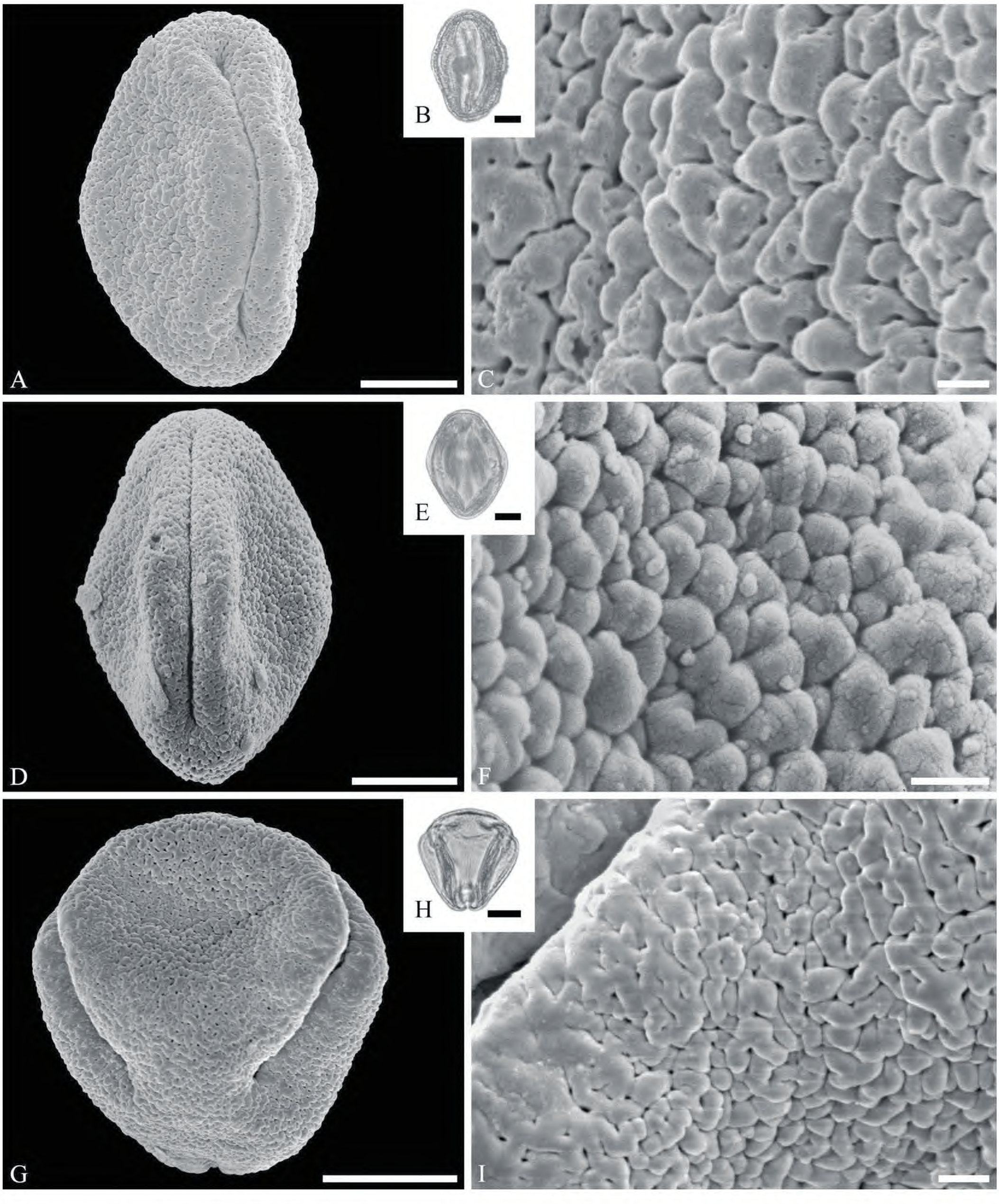


Figure 6. Cornalean pollen from the Early Paleocene of Greenland (A–C), the Middle Eocene of Germany (D–F), and Middle Eocene of Greenland (G–I). A–C. *Mastixia* Blume sp. from Agatdalen, Greenland. —A. SEM, equatorial view. —B. LM of the same grain showing tricolporate morphology, equatorial view. —C. Close-up showing sculpturing in area of mesocolpium. D–F. *Mastixia* sp. from Stolzenbach, Germany. —D. SEM, equatorial view. —E. LM of same grain showing tricolporate morphology, equatorial view. —F. Close-up showing sculpturing in area of mesocolpium. G–I. *Nyssa* L. sp. oblate pollen grain from Hareøen, Greenland. —G. SEM, oblique view. —H. LM of same grain, oblique view. —I. Close-up showing sculpturing along colpus and in area of mesocolpium. Scale bars: A, B, D, E, G, H = 10 μm; C, F, I = 1 μm.

The fossil record of Lecythidiaceae includes species related to *Barringtonia* J. R. Forst. & G. Forst. In addition to records based on woods from the latest Cretaceous (Late Maastrichtian) Deccan Traps of India accepted by Martínez-Millán (2010), we recognize pollen of *Barringtonia* morphology from the Middle Eocene of Stolzenbach, Germany (Fig. 7A–C). Pollen of this genus is distinctive and readily

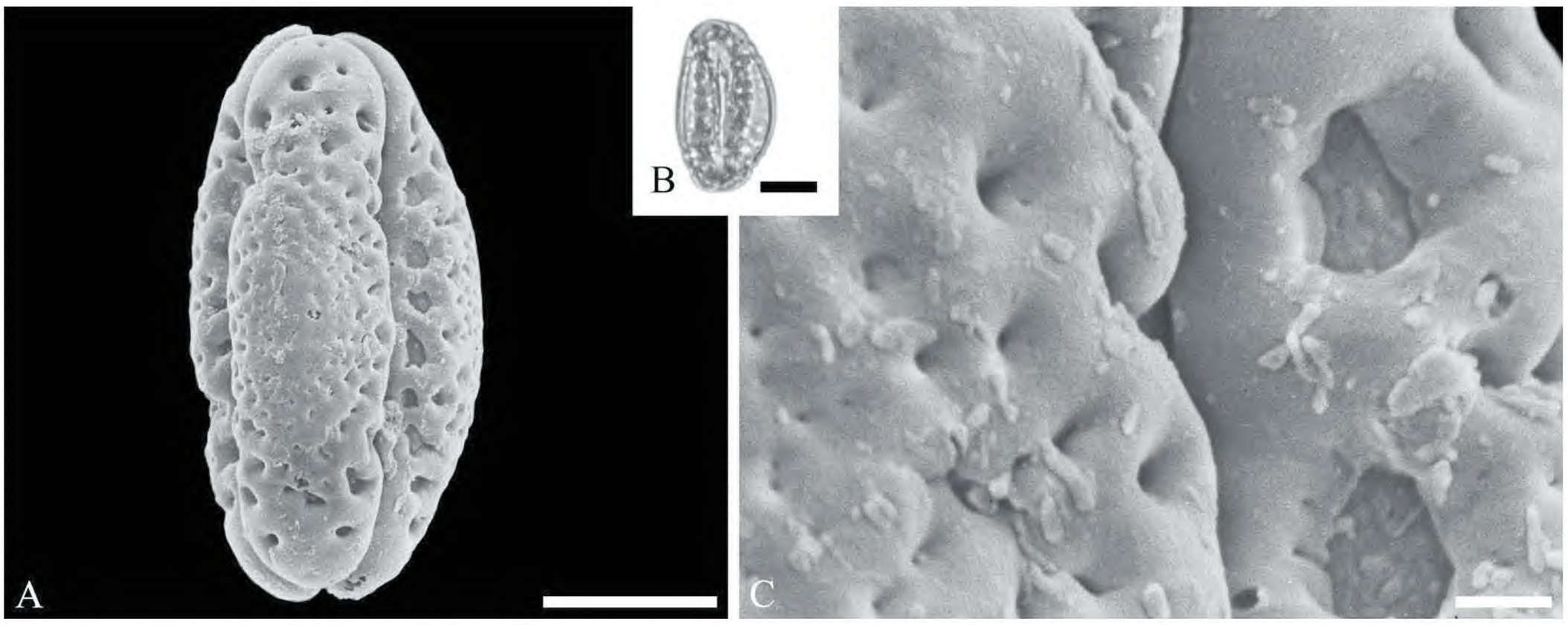


Figure 7. Lecythidiaceae pollen from the Middle Eocene of Stolzenbach, Germany. A–C. *Barringtonia* J. R. Forst. & G. Forst. sp. —A. SEM, equatorial view. —B. LM, equatorial view. —C. Close-up showing microreticulate, perforate sculpturing around colpus. Scale bars: A, B = 10 μm; C = 1 μm.

identifiable because of a unique reticulate type of sculpturing around the apertures (e.g., Muller, 1973). The Polemoniaceae are known from a single, quite complete fossil specimen of a plant with the roots, leaves, and fruits intact from the Middle Eocene of Utah, U.S.A. (Lott et al., 1998). The small stature and divided leaves of the single available specimen suggest that it was a small shrub perhaps adapted to subarid conditions.

Pollen of Sapotaceae is sufficiently distinctive (e.g., Harley, 1991) to be recognized as dispersed fossil grains. Examples of the tri-, tetra-, and sometimes stephano(5)-colporate grains are known from the Paleocene to the Miocene (e.g., Muller, 1981). However, the distinctive microornamentation necessary to confirm the fossil pollen identifications requires SEM as well as LM (e.g., Kmenta & Zetter, 2013). We document an example from the Eocene of Stolzenbach, Germany (Fig. 8A–F). In addition, twigs with attached leaves and fruits exhibiting caulofructy, a common feature of the family, are known from the Late Oligocene of Rott, Germany: *Sideroxylon salicites* (Weber) Weyland (Weyland, 1937; Winterscheid, 2006). Identification of the leaf attributed to *Chrysophyllum* by Mehrotra (2000), from the Paleocene of India, discussed above, does not seem convincing. Sapotaceae have seeds with a broad and distinctive hilar scar that would be easy to recognize in the fossil record, but there are very few records of them. The fossil genus *Sapotispermum* Reid & Chandler, accommodating seeds with morphology characteristic of the family, has been recognized from the Early Eocene London Clay (Reid & Chandler, 1933) and Middle Eocene of Germany (Collinson et al., 2012). One of the fossil seed types attributed to this family by Manchester (1994), as *Bumelia? globosa* from the Middle Eocene of Oregon, was later transferred to the unrelated genus *Sargentodoxa* Rehder & E. H. Wilson (Manchester, 1999).

Ebenaceae are difficult to confirm in the fossil record from leaves because they lack distinctive features, but the pollen has a distinctively organized microrugulate sculpturing, visible by SEM, that facilitates recognition of the genus *Diospyros*, e.g., from the Late Eocene of Florissant, Colorado (Fig. 8G–I). *Austrodiospyros cryptostoma* Basinger & Christophel 1985 was established based on on flowers with in situ pollen from Late Eocene of Anglesea, Victoria, Australia.

Actinidiaceae are a small family today but well represented in the fossil record, with flowers and fruits of *Parasaurauia allonensis* Keller, Herendeen & Crane 1996 and *Glandulocalyx* Schönenberger, Balthazar, Takahashi, Xiao, Crane & Herendeen 2012 from the Late Cretaceous (Late Santonian) of Georgia and seeds of *Actinidia* Lindl. from the Eocene of Oregon (Manchester, 1994) and Miocene sites of Europe (reviewed by Martínez-Millán, 2010). *Paradinandra suecica* Schönenberger & Friis is a flower from the Late Santonian–Early Campanian of Åsen, Scania, southern Sweden, with affinities to Actinidiaceae/Theaceae (Schönenberger & Friis, 2001). A seed named *Saurauia antiqua* Knobloch & Mai 1986 from the Late Turonian to Maastrichtian of Germany has also been accepted as a representative of this family (Friis et al., 2011).

Diapensiaceae are rare in the fossil record, but the flower described as *Actinocalyx bohrii* Friis 1985 from the Late Cretaceous (Late Santonian–Early Campanian) of Åsen, Scania, southern Sweden, has been accepted as a representative of this family (Martínez-Millán, 2010).

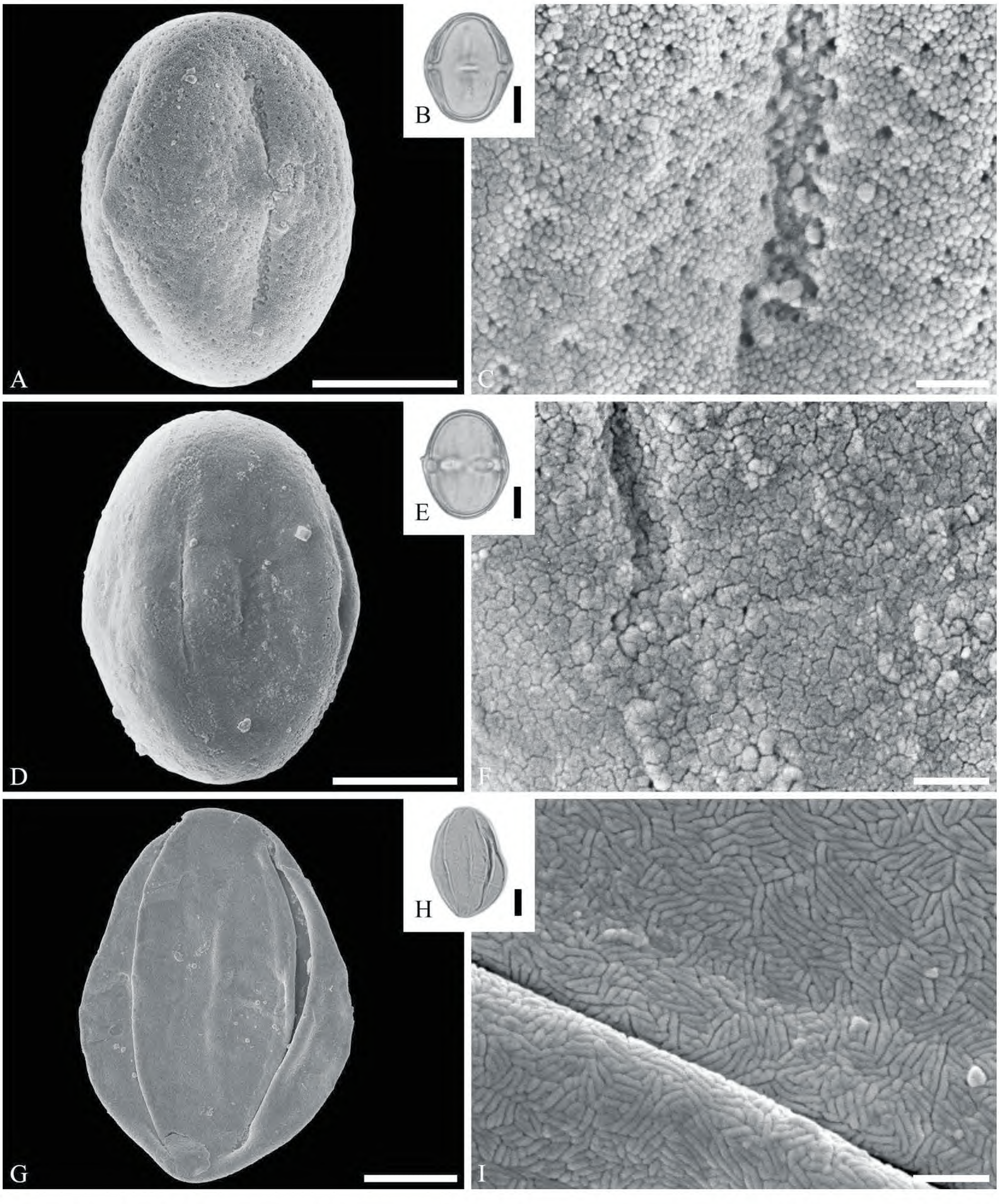


Figure 8. Ericales. Sapotaceae and Ebenaceae pollen. A–F. Sapotaceae pollen from the Middle Eocene of Stolzenbach, Germany. A–C. Sapotaceae gen. et spec. indet. —A. SEM, equatorial view. —B. LM of the same grain showing tricolporate morphology, equatorial view. —C. Close-up showing microverrucate and perforate sculpturing of mesocolpium and microverrucate and microechinate colpus membrane. D–F. Another sapotaceous grain. —D. SEM, equatorial view. —E. LM of the same grain, equatorial view. —F. Close-up showing fossulate and granulate sculpturing of mesocolpium. G–I. *Diospyros* pollen from the Late Eocene of Florissant, Colorado, U.S.A. —G. SEM, equatorial view. —H. LM of the same grain showing apparently psilate exine and elongate colpi. —I. Close-up showing fine ornamentation pattern. Scale bars: A, B, D, E, G, H = 10 μm; C, F, I = 1 μm.

The Theaceae are exemplified by fruits, winged seeds, and associated leaves from the Eocene of Tennessee, U.S.A. (Grote & Dilcher, 1989, 1992). Fossil leaves called *Ternstroemites* E. W. Berry, from the same strata, show the theoid tooth type characteristic for Ericales; because these leaves are found in the same sediments as unequivocal fruits and seeds of Theaceae, we think their assignment to Theaceae is probably correct.

Styracaceae. Although *Rehderodendron* Hu is restricted to Southeast Asia today, it was common in the Eocene to Miocene of Europe. Especially well-preserved examples are known from the Tertiary of Europe (Mai, 1970; Manchester et al., 2009a) including fruits of *R. stonei* (Reid & Chandler) Mai from the London Clay (Mai, 1970; Manchester et al., 2009a) and Sabals d'Anjou, France (Vaudois-Mieja, 1983) and *R. ehrenbergii* (Kirchheimer) Mai in the Miocene of Germany (Mai, 1970; Manchester et al., 2009a). The fruits are distinctive by the presence of three central locules surrounded by lacunose endocarp. Epigynous winged fruits with the intramarginal vein diagnostic of *Halesia* J. Ellis ex L. are known from the Pliocene of Europe (Tralau, 1965b) but have not been confirmed yet from older strata. *Styrax* L. is well represented by distinctive seeds, especially in the European Miocene (Kirchheimer, 1957).

Symplocaceae, with two extant genera, *Symplocos* Jacq. and *Cordyloblaste* Hensch. ex Moritzi, have an excellent fossil record of endocarps, reviewed by Mai and Martinetto (2006), Manchester and Fritsch (2014), and Fritsch et al. (2015). The oldest known occurrences are from the Early Eocene of London Clay. Pollen of *Symplocos* is usually triporate to tricolporate and oblate with rounded-triangular outlines. Pollen varies considerably in wall structure and surface ornamentation among species of *Symplocos*, including some types that seem very distinctive (e.g., Nagamasu, 1989). Krutzsch (1989) provided a critical review of fossil records previously attributed to Symplocaceae based mainly on LM, rejecting some and accepting others, but without indicating the precise characters upon which his judgments were based. Combined SEM and LM investigation were used to confirm the presence of three morphotypes of *Symplocos* pollen from the Upper Oligocene/Lower Miocene of Altmittweida, Saxony, Germany (Kmenta & Zetter, 2013).

Ericaceae. Pollen of Ericaceae in permanent tetrads is easily recognized from localities in Paleocene and younger strata. Here, we illustrate examples from the Early Paleocene of Agatdalen, Greenland (Fig. 9A–C), and the Middle Eocene of Profen, central Germany (Fig. 9D–F). Similar tetrads, as old as Middle Eocene (Geiseltal, Germany), can be identified to *Rhododendron* L. based on the presence of viscin threads (Zetter & Hesse, 1996). These pollen records augment the reports of dispersed seeds of *Rhododendron* from the Paleocene of England (Collinson & Crane, 1978) and Late Eocene of California, U.S.A. (Wang & Tiffney, 2001).

Clethraceae. Pollen of Clethraceae have often been described from the palynological record using LM only (e.g., Muller, 1981), and therefore many of the so-called Clethraceae pollen really belong to other families, e.g., Rosaceae, Cyrillaceae, and Actinidiaceae. Until now no convincing evidence based on combined LM and SEM studies exists showing the presence of "true" Clethraceae pollen.

Basal Lamiids and Garryales

Among basal Lamiids, the Icacinaceae are particularly well represented in the fossil record. Early records based on endocarps extend back to the Late Cretaceous (e.g., Knobloch & Mai, 1986), but these have not been fully confirmed with anatomical details. Endocarps placed in the modern genus *Phytocrene* Wall. by Scott and Barghoorn (1957) from the Turonian of New York, U.S.A., are anatomically distinct from that modern genus and probably are not Icacinaceae (Stull et al., 2012). By the Paleocene (~58 Ma), the family is known from a diversity of well-preserved and distinctive fruits resembling extant Icacinaceae (Pigg et al., 2008) and even the extant tribe Phytocreneae (Stull et al., 2012). Both extinct and extant genera of Icacinaceae are recognizable on the basis of fruits in the Middle Eocene (Manchester, 1999; Rankin et al., 2008; Collinson et al., 2012).

Pollen unique to *Platea* Blume, distinguished by tricolporate grains with a distinctive geometric pattern of crotonoid clavate (reticulum cristatum) ornamentation, is known from the Paleocene of North America (Lobreau-Callen & Srivastava, 1974) and Greenland (Fig. 10A–D). Lobreau-Callen and Srivastava (1974) documented the similarity with extant grains with comparative electron microscopy. Grains of *Platea* are very distinctive, even in the context of other Icacinaceae, which mostly have echinate ornamentation and colpate to porate apertures. A widespread Paleogene echinate and triporate pollen type, referred to by the misleading generic name *Compositoipollenites rhizophorus* (R. Potonié) R. Potonié, is believed to represent Icacinaceae (detailed SEM illustrations here in Fig. 10E–G and in Hofmann et al., 2011). These resemble the echinate type of pollen characteristic

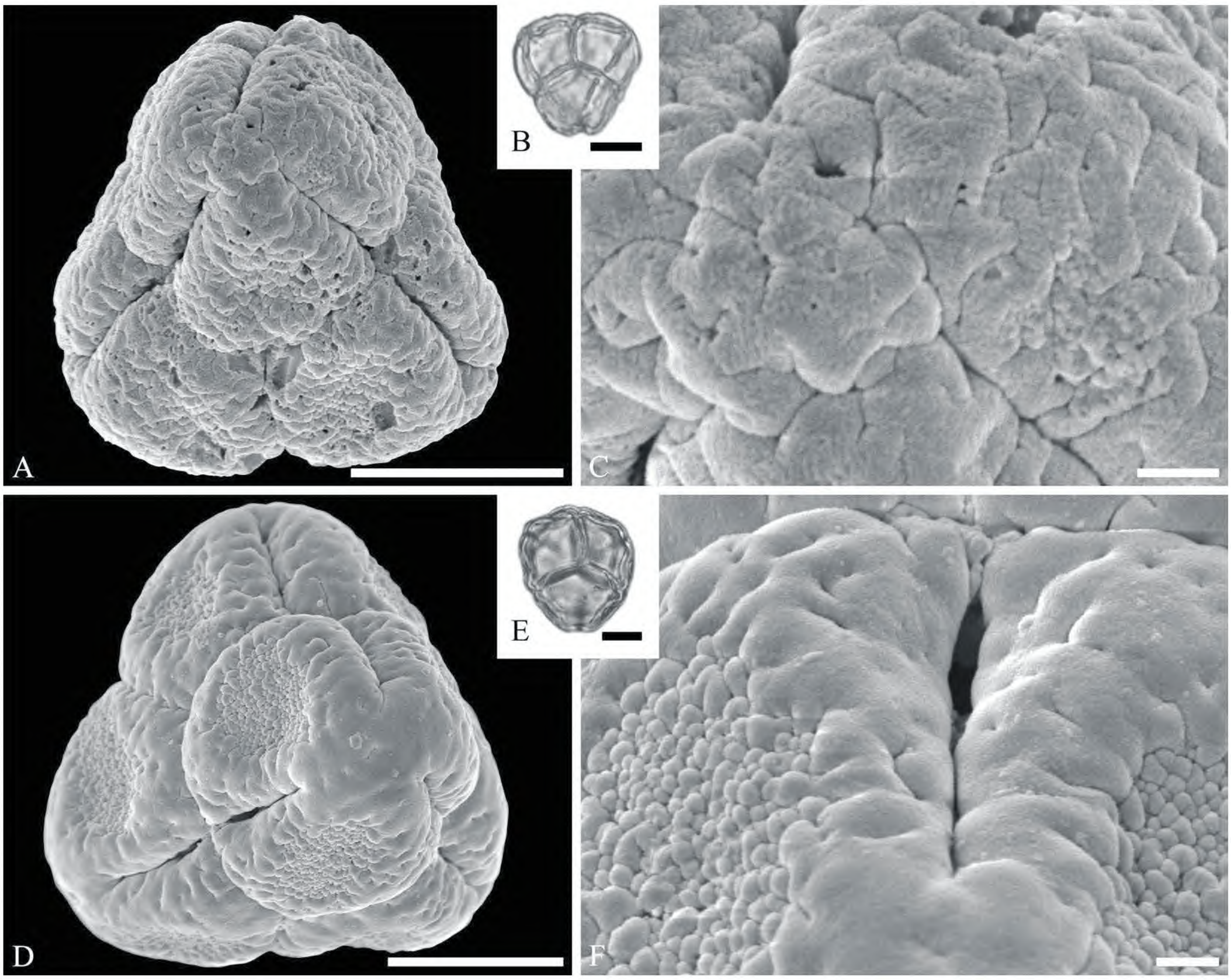


Figure 9. Ericaceae pollen from the Early Paleocene of Agatdalen, Greenland (A–C), and the Middle Eocene of Profen, central Germany (D–F). A–C. Ericaceae gen. et spec. indet. —A. SEM, tetrad in oblique view. —B. LM of the same tetrad in equatorial view. —C. Close-up showing fossulate and microstriate sculpturing in polar area. D–F. Ericaceae gen. et spec. indet. —D. SEM, tetrad in polar view. —E. LM of the same tetrad in equatorial view. —F. Close-up showing microverrucate sculpturing in central mesocolpium, and fossulate, perforate, and granulate sculpturing around colpi. Scale bars: A, B, D, E = 10 μm; C, F = 1 μm.

today of tribe Phytocreneae and some other representatives of the family (Lobreau-Callen, 1973), although a comprehensive comparative investigation remains to be done with attention to other families that also share echinate pollen.

Eucommiaceae are confirmed by their diagnostic pollen in Paleocene (Fig. 11A–C) (Zetter et al., 2011) and younger strata, e.g., the Middle Eocene of West Greenland (Fig. 11D–F). The tricolporate pollen is distinctive by its psilate surface (as seen in LM) with evenly distributed microechini as seen by SEM. Fruits of *Eucommia* Oliv. occur in the Early and Middle Eocene in Asia and North America. The North American occurrences range from Oligocene of southern Mexico (Magallón-Puebla & Cevallos-Ferriz, 1994) to the middle latitudes, e.g., Tennessee, Montana, and Oregon in the United States (Call & Dilcher, 1997), and in Miocene and younger strata of Europe and Asia (reviewed by Manchester et al., 2009a).

Solanales

The Solanales are conspicuous in lacking well-documented fossils, but new evidence of a *Physalis*-like fruit from the Eocene of Argentina has emerged (Wilf, 2013). The fossil record of Solanaceae was reviewed recently, and many fossils once attributed to this family have been discredited (Millán & Crepet, 2014).

Martin (2001) surveyed modern pollen of the Convolvulaceae as a basis for identifying fossil representatives based primarily on LM. She considered that the large tricolpate pollen species known as *Perfotricolpites digitatus* González Guzmán is similar to extant *Convolvulus* L. and *Operculina* Silva Manso, among other extant genera, and that a

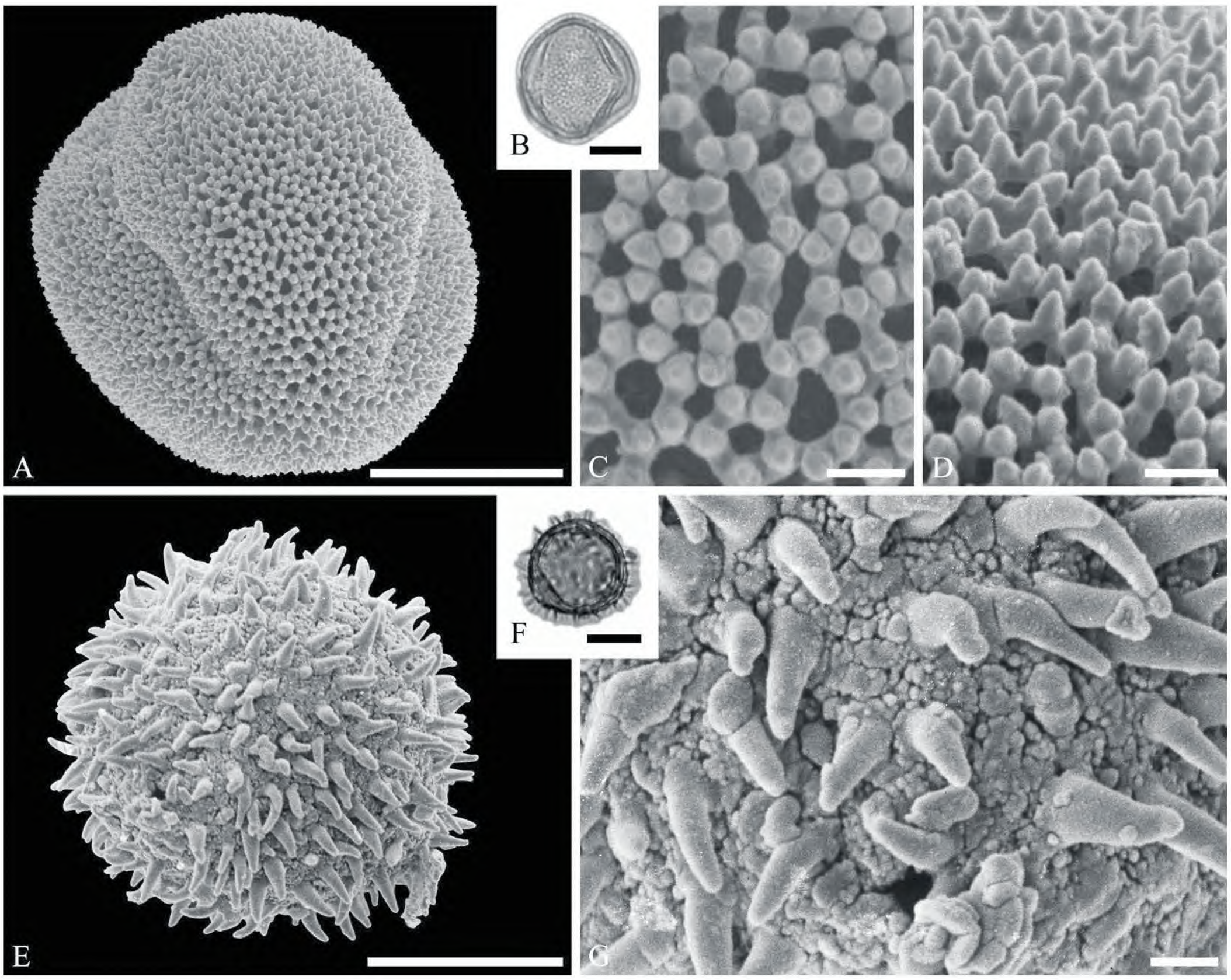


Figure 10. Icacinaceae pollen from the Early Paleocene of Agatdalen, Greenland (A–D), and Middle Eocene of Stolzenbach, Germany (E–G). A–D. *Platea* Blume sp. —A. SEM, equatorial view. —B. LM of same grain, equatorial view. —C. Close-up showing reticulum cristatum sculpture type in central mesocolpium. —D. Close-up showing reticulum cristatum sculpture type in polar area. E–G. Icacinaceae gen. et spec. indet. (*Compositoipollenites rhizophorus*). —E. SEM, polar view. —F. LM, polar view. —G. Close-up showing porus and surrounding echinate sculpturing. Scale bars: A, B, E, F = 10 μm; C, D, G = 1 μm.

small tricolpate grain known as *Tricolpites trioblatus* Mildenh. & Pocknall may be related to extant *Wilsonia* R. Br. and possibly *Cressa* L. Both of these pollen types occur in the Late Eocene of southern Australia. Other examples include the pantoporate species *Calystegiapollis microechinatus* Sal.-Cheb. (similar to *Calystegia* R. Br.) and *Xenostegia tridentata* (L.) D. F. Austin & Staples from the Early Eocene of Africa and *Perfotricolpites digitatus* from the Middle Eocene of Brazil. *Wilsonia*, now endemic to Australia, was in New Zealand in the Middle to Late Miocene (Martin, 2001).

In addition to the records mentioned above, we document the convolvulaceous genus *Merremia* Dennst. ex Endl., from the Middle Eocene of Stolzenbach, Germany (Fig. 12A–C). This genus, recognized previously from the Middle Oligocene in San Sebastián, Puerto Rico (Graham & Jarzen, 1969), is readily recognized by its tricolpate pollen grains with markedly thick exine as seen in LM in combination with a microechinate and perforate sculpturing observed in SEM.

Gentianales

The report of a flower of Gentianaceae from the Eocene of Texas, U.S.A. (Crepet & Daghlian, 1981), has not held up to close scrutiny. It was based on a single faintly preserved flower impression with seven well-developed apically pointed tepals and a central area with remnants of stamens containing well-preserved pollen of the extinct genus *Pistillipollenites* Rouse. Similar gemmate pollen occurs in some Gentianaceae but is not limited to that family. Stockey and Manchester (1988) described another specimen, better preserved, but apparently of the same kind of flower, also bearing in situ *Pistillipollenites* pollen, from the Middle Eocene of Horsefly, British Columbia. It is similar to the Texas flower in size and perianth configuration, but shows six rather

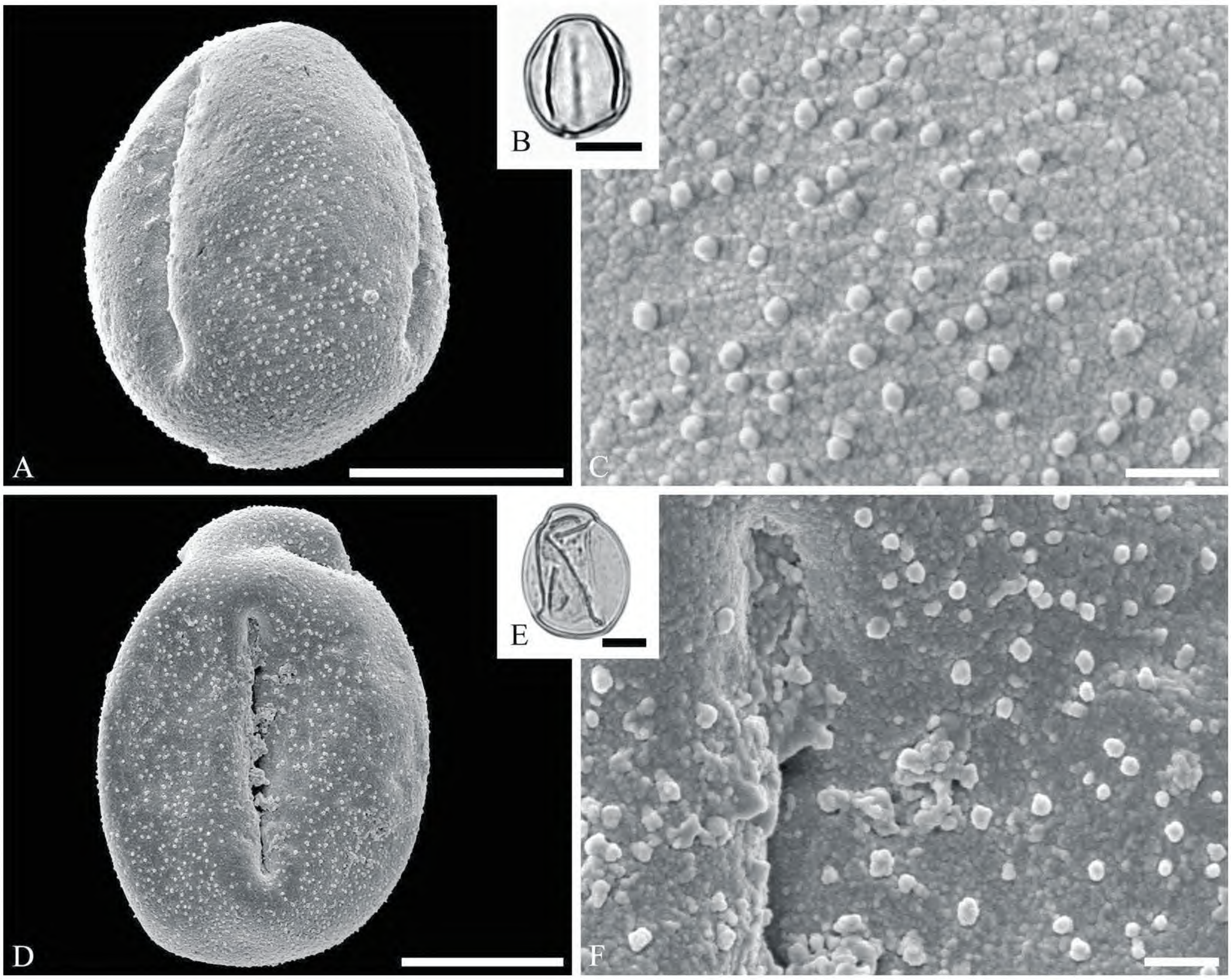


Figure 11. *Eucommia* Oliv. pollen from the Late Paleocene of Almont, North Dakota, U.S.A. (A–C), and from the Middle Eocene of Hareøen, western Greenland (D–F). A–C. *Eucommia* sp. —A. SEM, equatorial view. —B. LM of the same grain, equatorial view. —C. Close-up showing granulate and microechinate sculpturing of mesocolpium. D–F. *Eucommia* sp. —D. SEM, equatorial view. —E. LM of the same grain, equatorial view. —F. Close-up showing granulate and microechinate sculpturing of mesocolpium, and truncate polar end of colpus with microechinate colpus membrane. Scale bars: A, B, D, E = 10 mm; C, F = 1 μm.

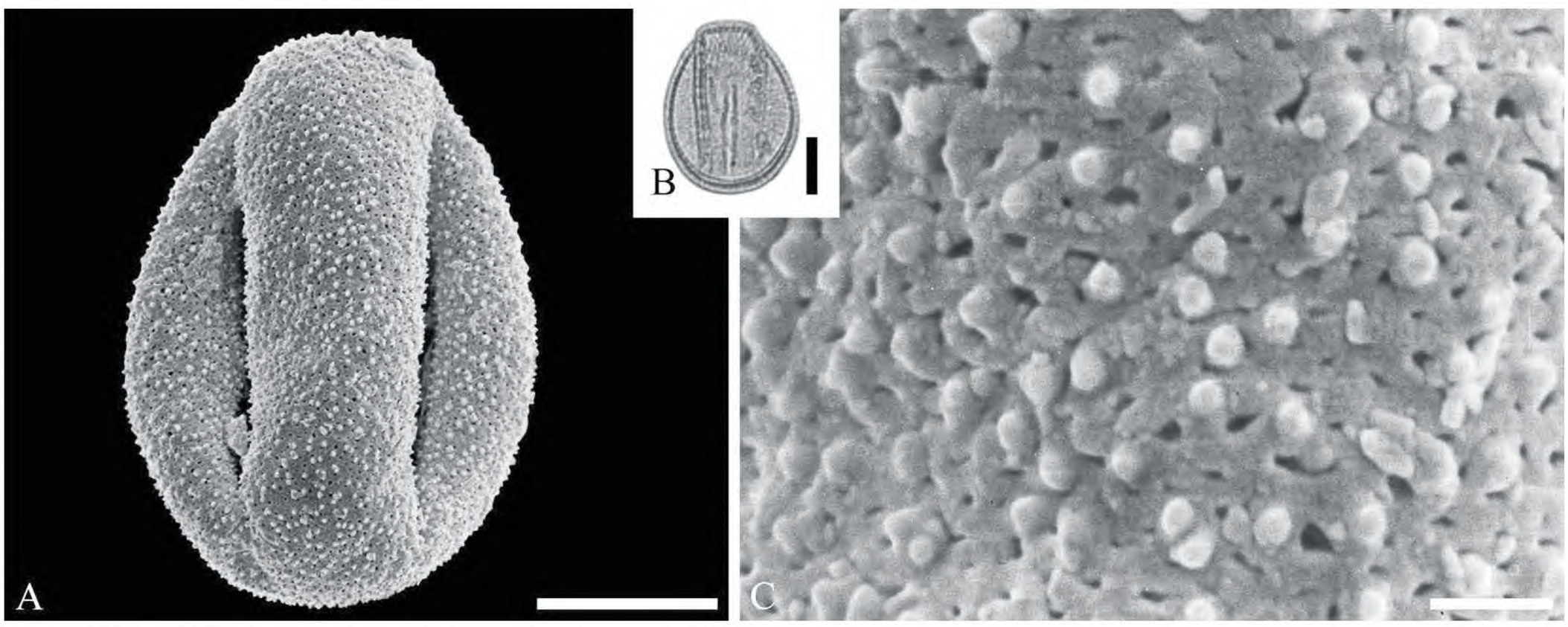


Figure 12. Convolvulaceae pollen from the Middle Eocene of Stolzenbach, Germany. A–C. *Merrermia* sp. —A. SEM, equatorial view. —B. LM, equatorial view. —C. Close-up showing microechinate, perforate sculpturing of mesocolpium. Scale bars: A, B = 10 μm; C = 1 μm.

than seven perianth lobes and a single anther opposite each lobe of the perianth (which they interpreted as calyx rather than corolla). The variability of six or seven sepals and the configuration of separate anthers is unlike the flowers of Gentianaceae, which have connate, basally attached stamens. As yet, the systematic affinities of these flowers, which are also present in the Paleocene and Eocene of Wyoming and North Dakota, referred to as *Calycites polysepala* Newberry (Manchester, 2014), are undetermined.

RUBIACEAE

Paleobotanical literature with reports of rubiaceous fossils was comprehensively reviewed by Graham (2008). There is a substantial fossil pollen record plus occasional megafossil reports. Graham indicated which records he considered acceptable and diplomatically indicated those that required more study as pending. A relatively early megafossil record of Rubiaceae is that of permineralized fruiting capsules named *Emmenopterys dilcheri* Manch. 1994 with intact winged seeds from the Middle Eocene of Oregon. The fossil was attributed to the modern genus *Emmenopterys* Oliv., but broader comparative work with modern genera in the same tribe Condamineeae sensu Kainulainen et al. (2010) would be desirable.

The leaves called *Paleorubiaceophyllum* Roth & Dilcher have not held up as Rubiaceae. The peculiar feature of those leaves is a foliar appendage commonly persisting at the base of the petiole on shed leaves. Roth and Dilcher (1979) compared these with the adnate stipules of some extant Rubiaceae, but in that family, including the genera they cited as most similar, each pair of stipules is fused and remains on the twig when the leaves abscise. A subsequently recovered fossil twig of *P. eocenicum* (Berry) Roth & Dilcher with attached leaves and intact stipules shows that the leaves were borne alternately rather than oppositely (UF-15738-27774), making it unlikely that this fossil is related to Rubiaceae. Roth and Dilcher (1979) successfully demonstrated that the species does not belong to *Leitneria* Chapm. or any of the other modern genera to which earlier investigators had assigned them; however, the true affinity of this plant, which was common at many sites in the Eocene of Tennessee and Mississippi, U.S.A., remains an interesting taxonomic puzzle.

LAMIALES S.L.

Within Lamiales s.l., the Oleaceae are well represented by samaroid fruits of *Fraxinus* L. starting in the Early and Middle Eocene of Asia and North America as reviewed by Call and Dilcher (1992). Pollen of Oleaceae are here documented from the Late Cretaceous (Early Campanian) of Elk Basin, Wyoming (Fig. 13A–C), and Middle Eocene Princeton Chert of British Columbia (Fig. 13D–F). Oleaceae pollen grains are mostly tricolporate, with very small and indistinct pori (Punt et al., 1991). Many Oleaceae pollen grains are also distinguished by their reticulate sculpturing and microechinate suprasculpture or segmented muri as seen in Figure 13. Combined SEM and LM have been used to identify grains as *Fraxinus*, *Olea*, and *Phillyrea* from Upper Oligocene/Lower Miocene sediments of Altmittweida, Saxony, Germany (Kmenta & Zetter, 2013). Wood of Oleaceae is traced to the latest Cretaceous Deccan Intertrappean Beds of India (Trivedi & Srivastava, 1981; Srivastava et al., 2015).

Seeds of Bignoniaceae appear in the Paleocene (Horiuchi & Manchester, 2011; Manchester, 2014), with small seeds somewhat similar to those of *Catalpa* Scop. (but not of that genus) from North America and Japan (~58 Ma). Large membranous seeds showing also the cordate outline of the cotyledons characteristic of Bignoniaceae from the Middle Eocene of Tennessee were attributed to a fossil genus, *Grotea* Wang, Blanchard & Dilcher 2013.

PEDALIACEAE

Trapella Oliv. has distinctive elongate, longitudinally ribbed fruits with a set of prominent laterally extended apical spines and has been identified in the Miocene and Pliocene of Europe (Tralau, 1964, 1965a).

ACANTHACEAE

Tripp and McDade (2014) scrutinized the published fossil record of this family. They were aware of 51 published reports and accepted several of the fossils as sufficiently convincing to use as basis for age-calibrating their phylogeny of the family. To assess the utility of fossils for divergence time estimates, Tripp and McDade (2014) tabulated the fossils according to their ranks of confidence in the taxonomic identifications and age estimates and then utilized only those that received high scores. Fossils that were recently accepted for this family include the seed of *Acanthus rugatus* Reid & Chandler 1926 from the latest Eocene Bembridge flora of England. Tripp and McDade (2014) expressed reservations about this, though noting that seed sculpture has not been exhaustively surveyed across tribe Acantheae. In addition, the seed is only known from its external

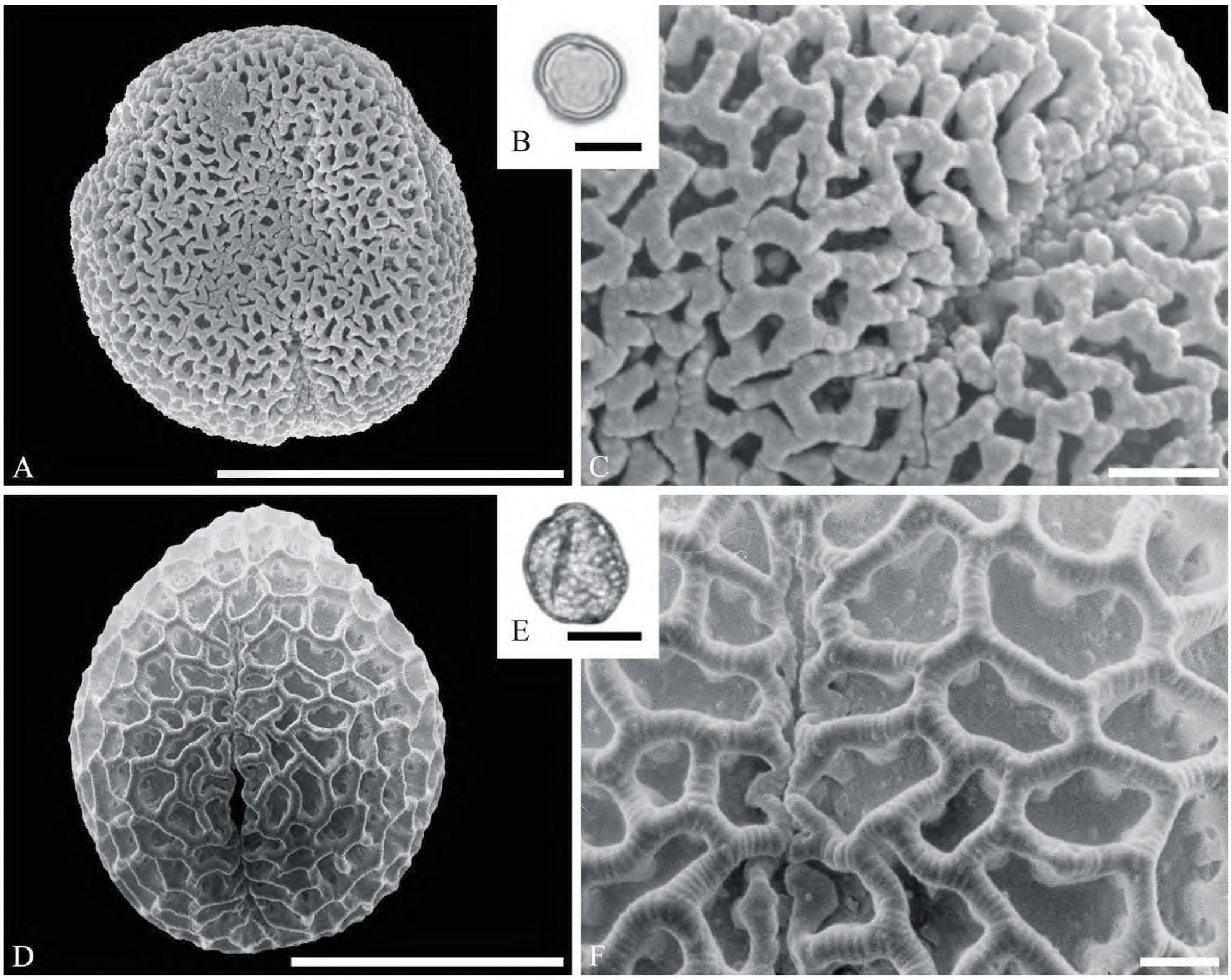


Figure 13. Oleaceae pollen from the Upper Cretaceous of Wyoming, U.S.A. (A–C), and the Middle Eocene of Princeton Chert, Canada (D–F). A–C. Oleaceae gen. et spec. indet. —A. SEM, polar view. —B. LM of the same grain, polar view. —C. Close-up showing reticulate sculpturing with microechinate suprasculpture and microechinate and microverrucate colpus membrane. D–F. Oleaceae gen. et spec. indet. —D. SEM, equatorial view. —E. LM, equatorial view. —F. Close-up showing segmented reticulate sculpturing. Scale bars: B, E = 10 μm; A, C, D, F = 1 μm.

characters, without other anatomical information, and it is not hard to imagine that this seed could represent an unrelated family.

Among the amazing diversity of pollen morphological types in extant Acanthaceae (Scotland & Vollesen, 2000), some are very distinctive and readily recognized in the fossil record. This allowed Tripp and McDade (2014) to support the published taxonomic placements of a fossil similar to *Hulemacanthus* S. Moore (Raj, 1961) from the Miocene of Nigeria, and *Areolipollis insularis* Mautino from the Upper Miocene of Mexico. The latter fossil is dicolporate with distinctive areoles that surround the germinal apertures, traits known only from Justicieae (Graham, 1988).

Lamiaceae and Verbenaceae fossil records are not very good. Reid and Chandler (1926) recognized the lamiaceous genera *Ajuginucula* Reid & Chandler and *Melissa* L., but the external structures preserved are not sufficiently distinctive to be fully confident about these determinations. Stephanocolpate (6-colpate) and doubly reticulate pollen consistent with Lamiaceae is recognizable, for example, from the Miocene of China (Fig. 14) and the Middle Miocene of Austria (Manchester, Grímsson & Zetter, pers. obs.).

Aquifoliales

Tricolporate pollen of *Ilex* L., with distinct clavate ornamentation, is readily recognizable in the fossil record, as exemplified by the specimens shown here from the Eocene of Profen, Germany (Fig. 15A–C), and the Miocene of Beipaizi, northeastern China (Fig. 15D–F). In a review of the widespread fossil pollen record for *Ilex*, Martin (1977) accepted many Eocene and younger records throughout the world. She also noted reports from the Cretaceous, but these remain to be well documented with SEM. An

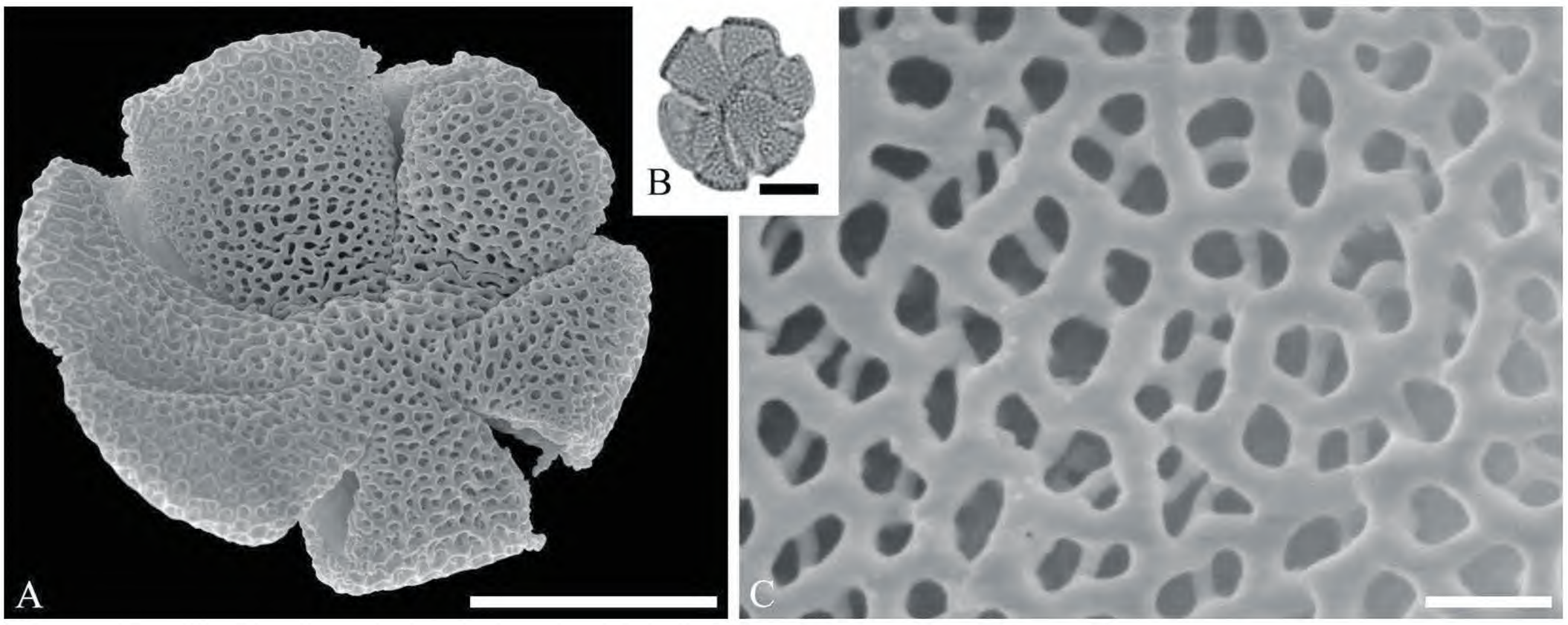


Figure 14. Lamiaceae pollen from the Middle to Late Miocene of Beipaizi, northeast China. A–C. Lamiaceae gen. et spec. indet. —A. SEM, polar view. —B. LM, polar view. —C. Close-up showing bireticulate sculpturing in mesocolpium. Scale bars: A, B = 10 μm; C = 1 μm.

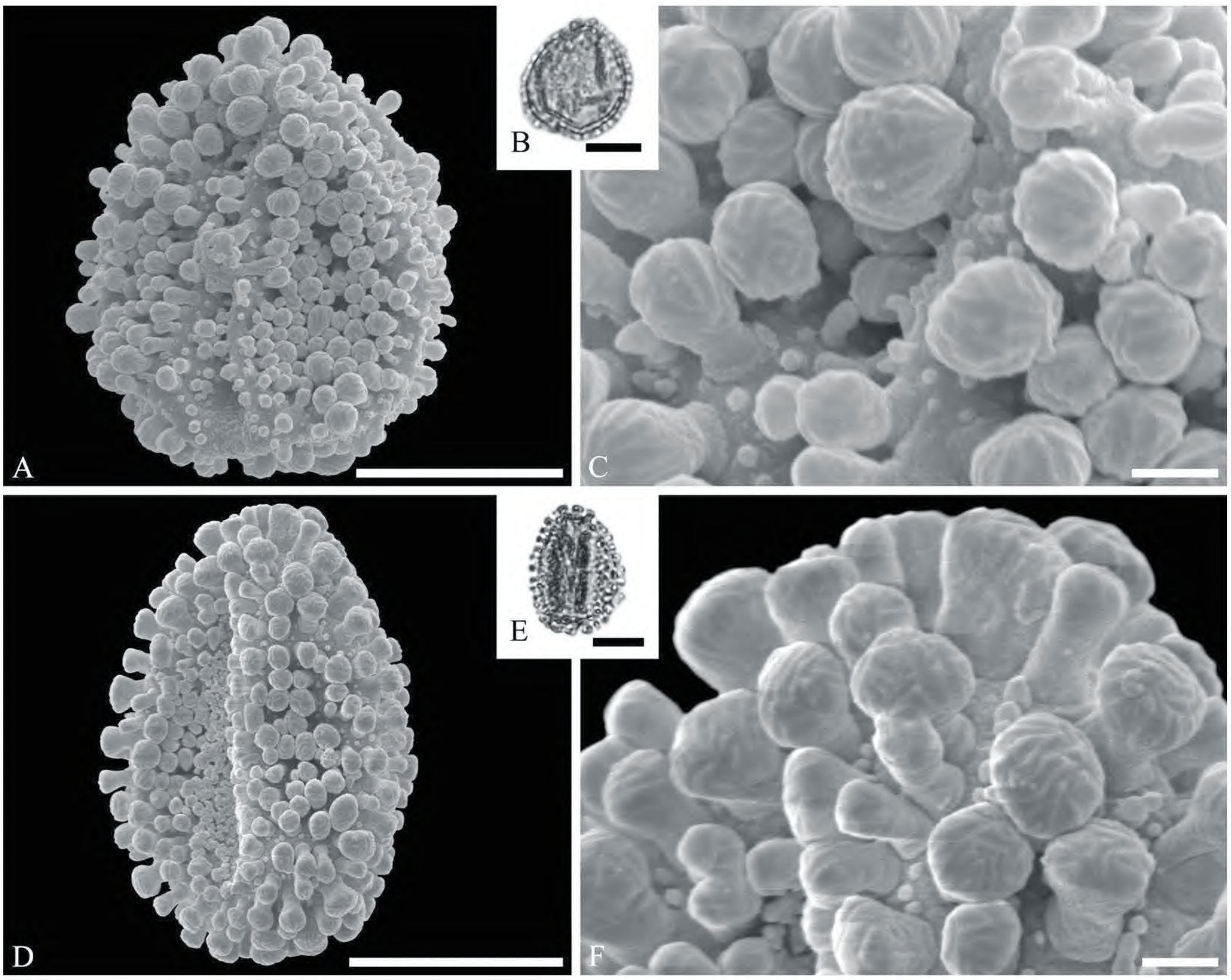


Figure 15. Aquifoliaceae. *Ilex* L. pollen from Middle Eocene of Profen, central Germany (A–C), and the Middle to Late Miocene of Beipaizi, northeast China (D–F). A–C. *Ilex* sp. —A. SEM, equatorial view. —B. LM, equatorial view. —C. Close-up showing clavate and microbacculate sculpturing in central mesocolpium. D–F. *Ilex* sp. —D. SEM, equatorial view. —E. LM, equatorial view. —F. Close-up showing clavate and microbacculate sculpturing in polar area. Scale bars: A, B, D, E = 10 μm; C, F = 1 μm.

example is *Ilexpollenites* F. Thiergart ex R. Potonié from the Late Cretaceous (Campanian to Maastrichtian) of California (Chmura, 1973).

ASTERALES

The fossil record of Campanulaceae is rather poor. In addition to the few seed records from Miocene reviewed and accepted by Martínez-Millán (2010), *Campanula* L. pollen, characterized by combined microrugulate and echinate ornamentation, has been documented by SEM and LM from the Pliocene of Iceland (Denk et al., 2011: pl. 10.14, figs. 1–3).

MENYANTHACEAE

In addition to the record of a *Menyanthes* L. seed from the Miocene in Nowy Sącz Basin, Poland (Łańcucka-Środoniowa, 1979), accepted by Martinez-Millan (2010), the family is recognized by pollen reviewed by Barreda et al. (2010). *Striasyncolpites laxus* Mildenh. & Pocknall pollen (illustrated by Barreda et al., 2010: pl. I, 1) is characterized by oblate, tricolporate, parasyncolpate grains with striate exine and greatly resembles the pollen of extant herbaceous genera *Villarsia* Vent. and *Liparophyllum* Hook. f. This pollen type occurs in the Oligocene and Miocene of New Zealand, Australia, and Patagonia, South America (Mildenhall & Pocknall, 1989; Macphail & Hill, 1994; Macphail, 1999; Zetter et al., 1999; Palamarczuk & Barreda, 2000; Barreda et al., 2010).

Goodeniaceae are also known best from dispersed pollen records extending back to the Oligocene. Grains of *Poluspissusites* Sal.-Cheb. are prolate to subprolate, tricolporate, with the exine thicker at poles, clearly stratified, with digitate infratectal columellae. They have general similarities to the *Scaevola–Goodenia* group and are known from the Oligocene of Cameroon (*P. digitatus* Sal.-Cheb.; Salard-Cheboldaeff, 1978) and Late Oligocene and Early Miocene of New Zealand (*P. ramus* Pocknall; Pocknall, 1982; Macphail, 1999) and Patagonia (*P. puntensis* Barreda; Barreda, 1997a, 1997b; Barreda et al., 2010: pl. I, 2), and Late Miocene–Pleistocene of Australia (*P. ramus*) (Macphail, 1999).

Calyceraceae pollen has been identified with studies using both SEM and LM from the Early and Late Miocene of Chubut Province, Argentina (Palazzesi et al., 2010). The small, tricolporate, subspheroidal to suboblate pollen grains, rhombic in equatorial view and subtriangular in polar view, are relatively distinctive. In addition, Palazzesi et al. (2010) note that the fossil grains have tectate, columellate exine and the nexine is thickened toward endoapertures, causing a distinctive wall protrusion on the external surface, similar to what is observed in modern pollen of the *Gamocarpha* DC. type of the Calyceraceae. According to Palazzesi et al. (2010: 239), these fossils "establish the presence of related species in the Miocene of southern South America. The first major radiation of this family occurred during a period of significant shift to more arid conditions that caused extinction of numerous Gondwanan elements but had little effect on the Calyceraceae."

ASTERACEAE

The fossil record of Asteraceae is best known from dispersed pollen records, and credible megafossil records have been few (Graham, 1996). However, the impression of a fossil capitulum, *Raiguenrayun cura* Barreda, Katinas, Passalia & Palazzesi, with multiseriate-imbricate involucral bracts and pappus-like hairs, recently described from the Eocene of Argentina (~47.5 Ma; Barreda et al., 2012), displays a set of morphological features today diagnostic of Asteraceae. The suite of characters seen in this fossil conform with those found in taxa considered phylogenetically close to the root of the family, "such as Stifftieae, Wunderlichioideae and Gochnatieae (Mutisioideae sensu lato) and Dicomeae and Oldenburgieae (Carduoideae), today endemic to or mainly distributed in South America and Africa" (Barreda et al., 2012: 127). Apart from the infructescence of *R. cura*, it seems strange that individual pappus-bearing achenes are not common in the fossil record; given their adaptation for wind dispersal, they should be preserved as impression fossils in lacustrine deposits.

The worldwide fossil record of the Asteraceae was reviewed by Graham (1996). Along with the literature review, he included images documenting pollen grains from Neogene sites in Panama, Mexico, and Haiti. Although most megafossil reports reviewed by Graham (1996) are dubious (including *Baccharites* G. Saporta leaves and *Cypselites* Heer fruits), many of the dispersed pollen records are convincing records for the family, and some can be assigned to specific clades based on pollen ornamentation, including representatives of *Ambrosia* L. type, Liguliflorae type, Lactuceae–Veronieae type, and *Mutisia* L. f. type. The early pollen records in Europe, North America, and India were recorded as Late Oligocene, and as Early Miocene in Africa, Australia, and East Asia. Asteraceae include a number of readily recognized pollen types, some with highly distinctive ornamentation. Here we

illustrate examples of asteraceous pollen from the Eocene Princeton Chert of British Columbia (Fig. 16A–C) and from the Miocene of China (Fig. 16D–I), documented by LM and SEM.

Different clades of Asteraceae can be recognized based on pollen morphology. Pollen grains characteristic of tribe Mutiseae have been recognized from the Late Paleocene–Eocene of South Africa, treated under the name *Tubulifloridites antipodica* Cookson ex R. Potonié (Zavada & de Villiers, 2000), and from the Early Oligocene of northwestern Tasmania, named *Mutisiapollis patersonii* Macphail & Hill 1994. Fossil pollen from these and other Southern Hemisphere sites have been used to recognize the Barnadesioideae and Nassauvieae, as well as Mutisieae (Barreda et al., 2008).

Apiales

Diversification times and biogeographic patterns have been reviewed recently for the Apiales (Nicolas & Plunkett, 2014). Nicolas and Plunkett estimated the origin of Apiales to have occurred in Australasia in the Early Cretaceous (~117 Ma). In their assessment, most major clades also appear to have originated in Australasia, with the youngest family (Apiaceae) having originated in the Late Cretaceous, ~87 Ma.

TORICELLIACEAE

Fruits diagnostic of the extant genus *Toricellia* DC. are known from the Late Paleocene (~58 Ma; Manchester et al., 2009a: figs. 53–56) as well as from the Middle Eocene of Oregon, U.S.A. (Manchester, 1999: 476, fig. 1), and Middle Eocene of Germany (Collinson et al., 2012). Newly obtained micro-computed tomography (micro-CT) scan data on Early Eocene London Clay fruits (courtesy of M. Collinson, 2014) indicate that the fossil species *Spondiaecarpon operculatum* Reid & Chandler actually corresponds to the extant genus *Toricellia* and is similar to the species *T. bonesii* (Manch.) Manch. from Oregon. The distinctive morphological characters of these fruits (both modern and fossil) were well documented by Meller (2006) in a study of well-preserved specimens from the Miocene of Germany.

ARALIACEAE

Pollen of Araliaceae, with combined characteristic features including thick exine, especially thickened around the apertures, microreticulate to reticulate or rugulate (perforate, fossulate) sculpturing under SEM, and often lalongate endoapertures, are known from the Late Cretaceous (Early Campanian) of Wyoming (Fig. 17A–C), Paleocene and Eocene of Greenland (Fig. 17D–F), and Eocene of western North America (Fig. 17G–I) and Europe (Profen, Germany; Manchester, Grímsson & Zetter, pers. obs.). Pollen assignable to the genus *Aralia* L., distinguished by its thickened exine in polar areas in combination with other general features of Araliaceae, is recognized from the Eocene Princeton Chert of British Columbia (Fig. 17G–I).

An Eocene fossil leaf species that was documented in anatomical detail and attributed to extant *Dendropanax* Decne. & Planch. (Dilcher & Dolph, 1970) and accepted as valid Araliaceae in some subsequent works (e.g., Martínez-Millán, 2010; Nicolas & Plunkett, 2014) needs to be reconsidered. The perfectly symmetrical and consistently lobed leaves do not match with *Dendropanax*, which is inconsistently lobed even on the same twig and tends to be longer than wide. We could not find a convincing match among any extant Araliaceae. Somewhat similarly lobed leaves are found in extant *Acanthopanax* (Decne. & Planch.) Miq., *Eleutherococcus* Maxim., and *Kalopanax* Miq., but those leaves are strongly serrate in contrast to the entire-margined leaves of the fossil. As Dilcher and Dolph (1970) pointed out, the fossil leaves differ significantly from modern *Dendropanax* by the consistently papillate lower epidermis with a single central papilla per cell in the fossil, which is not seen in any modern species of the genus. This feature has not been documented in any other modern Araliaceae either. Dilcher and Dolph (1970) were intrigued that the Tennessee fossils were consistently prominently lobed, whereas modern *Dendropanax* has lobed leaves mostly in juvenile condition, whereas mature leaves are unlobed. These are important differences that readily exclude the fossil from *Dendropanax*.

Despite the questionable Eocene leaf record of Araliaceae, there are fossil fruits from the Eocene that show the schizocarpic fruit type and persistent epigynous perianth, consistent with assignment to this family, such as *Paleopanax* Manch. from the Clarno Formation of Oregon (Manchester, 1994) and an unnamed specimen from the Middle Eocene Claiborne Formation of Tennessee (Fig. 18A).

APIACEAE

Fruits of Apiaceae are confirmed from the Late Cretaceous based on *Carpites ulmiformis* Dorf 1942 from the Maastrichtian of Montana and Wyoming (Manchester & O'Leary, 2010). This species is based on well-preserved, winged fruits with a persistent

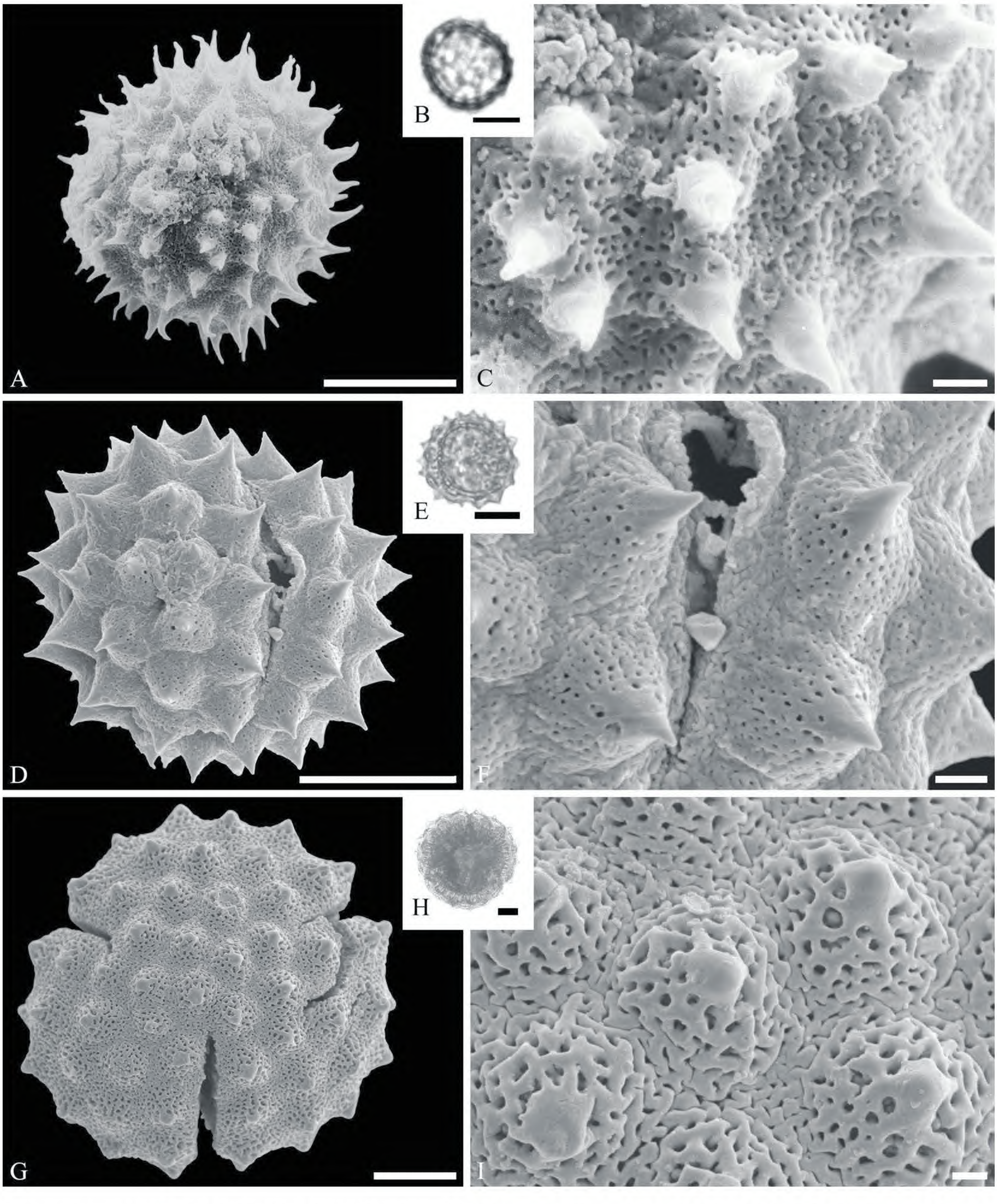


Figure 16. Asteraceae pollen from the Middle Eocene of Princeton Chert, British Columbia, Canada (A–C), and from the Middle to Late Miocene of Beipaizi, northeast China (D–I). A–C. Asteraceae gen. et spec. indet. —A. SEM, equatorial view. —B. LM. —C. Close-up showing echinate and perforate sculpturing in mesocolpium. D–F. Asteraceae gen. et spec. indet. —D. SEM, equatorial view. —E. LM, equatorial view. —F. Close-up showing echinate and perforate sculpturing in mesocolpium, and microverrucate colpus membrane around colporus. G–I. Asteraceae gen. et spec. indet. —G. SEM, polar view. —H. LM, polar view. —I. Close-up showing echinate and perforate sculpturing in polar area. Scale bars: A, B, D, E, G, H = 10 μm; C, F, I = 1 μm.

epigynous calyx of several equal, basally fused tepals; each disseminule has an elliptical fruit body with a pair of prominent lateral wings. Although *C. ulmiformis* does not conform precisely to a modern genus, the disseminules resemble mericarps of extant *Thapsia polygama* Desf. and *Astrotricha cordata* A. R. Bean as illustrated for comparison by Manchester and O'Leary (2010).

Apiaceous fruits have been recovered from the Eocene Green River Formation of Colorado. These

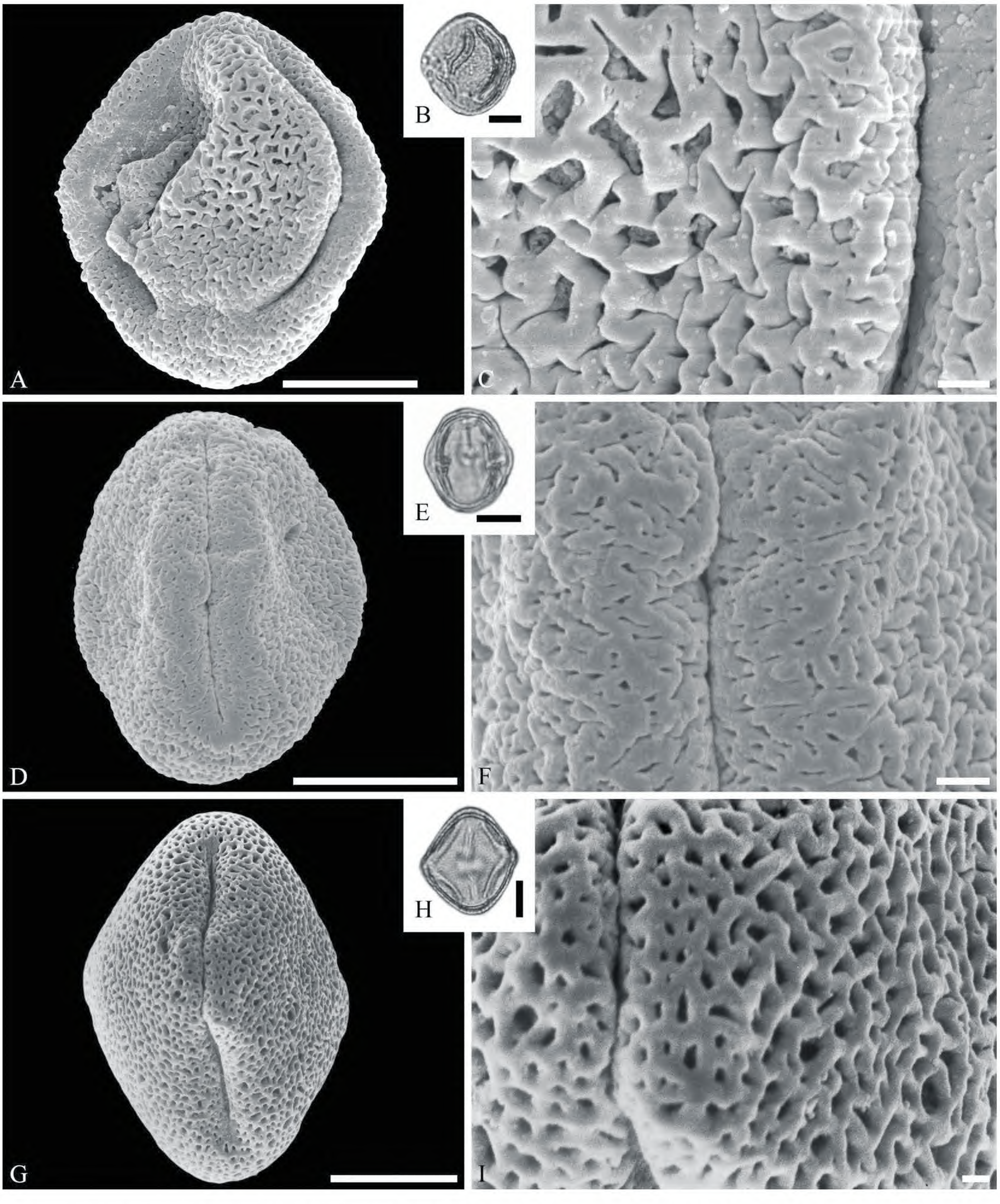


Figure 17. Araliaceae pollen from the Upper Cretaceous of Wyoming, U.S.A. (A–C), the Early Paleocene of Agatdalen, Greenland (D–F), and the Middle Eocene of Princeton Chert, Canada (G–I). A–C. Araliaceae gen. et spec. indet. —A. SEM, equatorial view. —B. LM, equatorial view. —C. Close-up showing reticulate to microreticulate sculpturing in mesocolpium. D–F. Araliaceae gen. et spec. indet. —D. SEM, equatorial view. —E. LM, equatorial view. —F. Close-up showing verrucate, fossulate, and perforate sculpturing. G–I. *Aralia* L. sp. —G. SEM, equatorial view. —H. LM, equatorial view. —I. Close-up showing microreticulate to reticulate sculpturing in mesocolpium. Scale bars: A, B, D, E, G, H = 10 μm; C, F, I = 1 μm.

are schizocarps with a stylopodium and peristent epigynous perianth (Fig. 18B, C). Apiaceae pollen is also recognizable in the fossil record. We illustrate examples from the Middle to Late Miocene of Beipaizi, northeast China (Fig. 19). The prolate tricolporate grains show a distinctive rugulate and striate to verrucate and fossulate ornamentation.

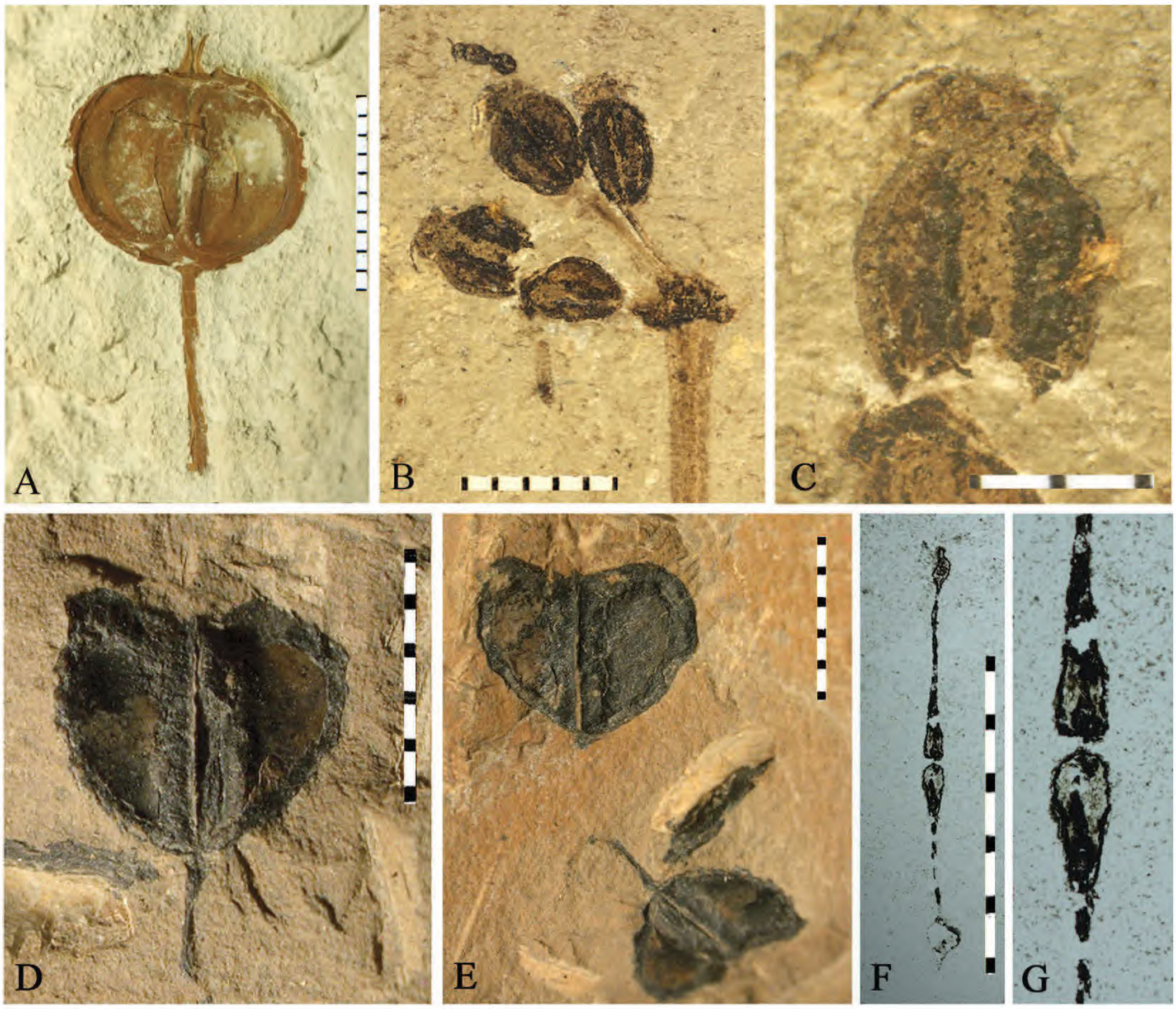


Figure 18. Araliales and cf. Araliales. —A. Fruit of Araliaceae from the Middle Eocene Puryear clay pit of the Claiborne Formation, Tennessee, U.S.A. Note apical styles arising from an apical depression surrounded by perianth rim (UF-15820-53151). —B. Apiaceous umbel from early Middle Eocene Green River Formation of Colorado, U.S.A. (UF-584-58340). —C. Detail of fruit from B showing stylopodium and recurved styles. D–F. The oldest potential Araliales fossil, *Araliaecarpum kolymensis* Samylina from the Early Cretaceous (Albian) Buor-Kemiusskaja locality of eastern Siberia. —D, E. Pedicellate fruits in face view. Perianth position unclear (BIN 396-2). —F. Fruit in transverse section showing what appear to be two mericarps. —G. Enlargement of mericarps from F. Scale bars: A = 1 cm; B, D–F = 0.5 cm; C = 2 mm.

Araliaecarpum

The oldest potential Araliales fossil, and indeed the oldest potential Asterid, is the fruit called *Araliaecarpum kolymensis* Samylina from the Early Cretaceous (Albian) Buor-Kemiusskaja locality near the Zyrianka River in eastern Siberia (Samylina, 1960). The fruit is about 6 mm long and is syncarpous with two carpels borne on a thin pedicel (Fig. 18D, E). It is possible that the fruits were schizocarpic as suggested by transverse section (Fig. 18F, G). An important question is whether this fruit developed from an epigynous flower as expected in Apiales, or from a hypogynous one. If hypogynous, then some Malvid families, e.g., Brassicaceae and Sapindaceae, might come into consideration. There is no obvious swelling at the junction of the fruit and pedicel that would be interpreted as the position of a hypogynous perianth. On the other hand, there is not any obvious perianth bulge or scar at the apical side of the fruit. More detailed anatomical comparative work is needed to assess whether the resemblance to Apicaceae is more than a superficial one.

Dipsacales

Adoxaceae

Pollen of *Viburnum* L. can be quite distinctive and sometimes readily recognizable down to particular sections/clades (e.g., Donoghue, 1985) when studied by both SEM and LM. Augmenting

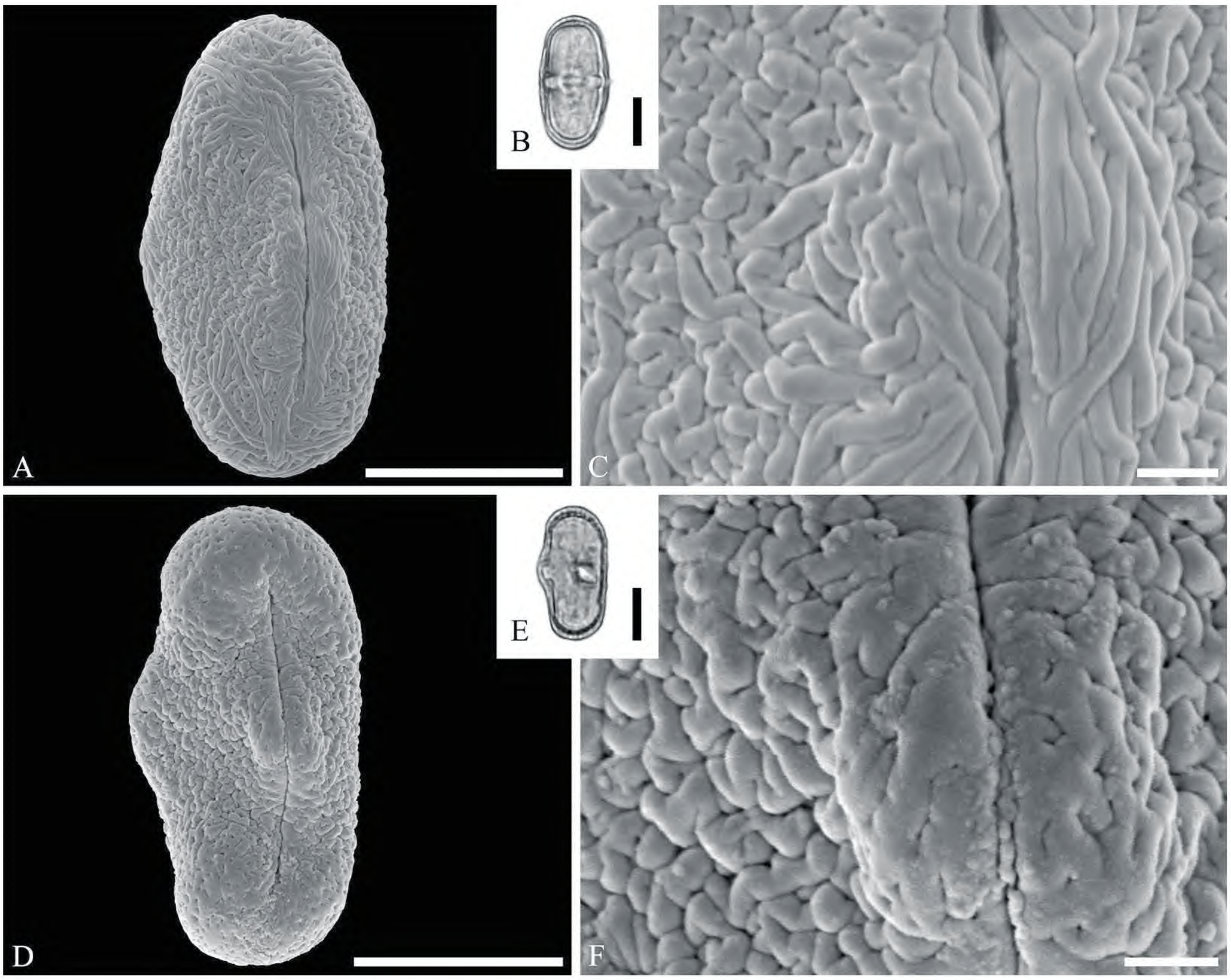


Figure 19. Apiaceae pollen from the Middle to Late Miocene of Beipaizi, northeast China. A–C. Apiaceae gen. et spec. indet. —A. SEM, equatorial view. —B. LM, equatorial view. —C. Close-up showing rugulate and striate sculpturing in mesocolpium. D–F. Apiaceae gen. et spec. indet. —D. SEM, equatorial view. —E. LM, equatorial view. —F. Close-up showing rugulate to verrucate and fossulate sculpturing in mesocolpium. Scale bars: A, B, D, E = 10 mm; C, F = 1 μm.

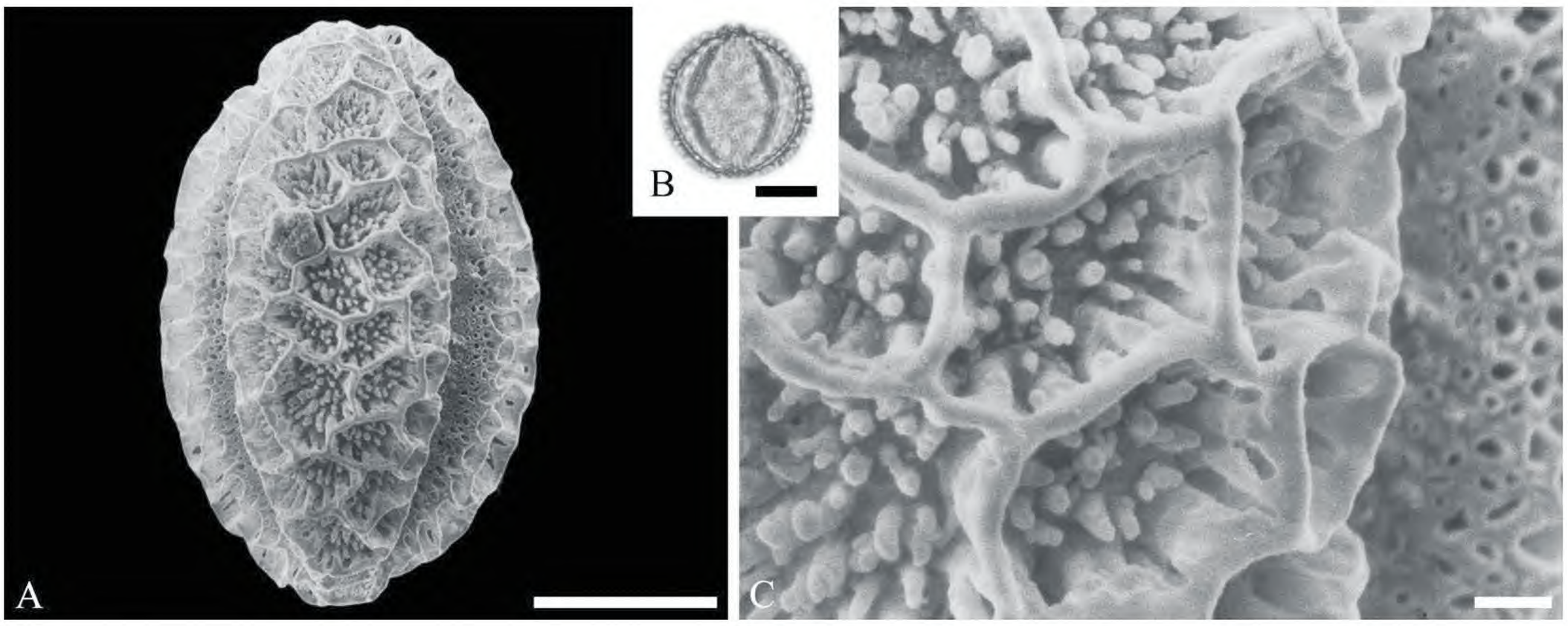


Figure 20. *Viburnum* L. (Caprifoliaceae) pollen from the Middle Eocene of Princeton Chert, Canada (A–C). —A. SEM, equatorial view. —B. LM, equatorial view. —C. Close-up showing reticulate sculpturing in mesocolpium, and lumina with numerous freestanding columellae. Scale bars: A, B = 10 μm; C = 1 μm.

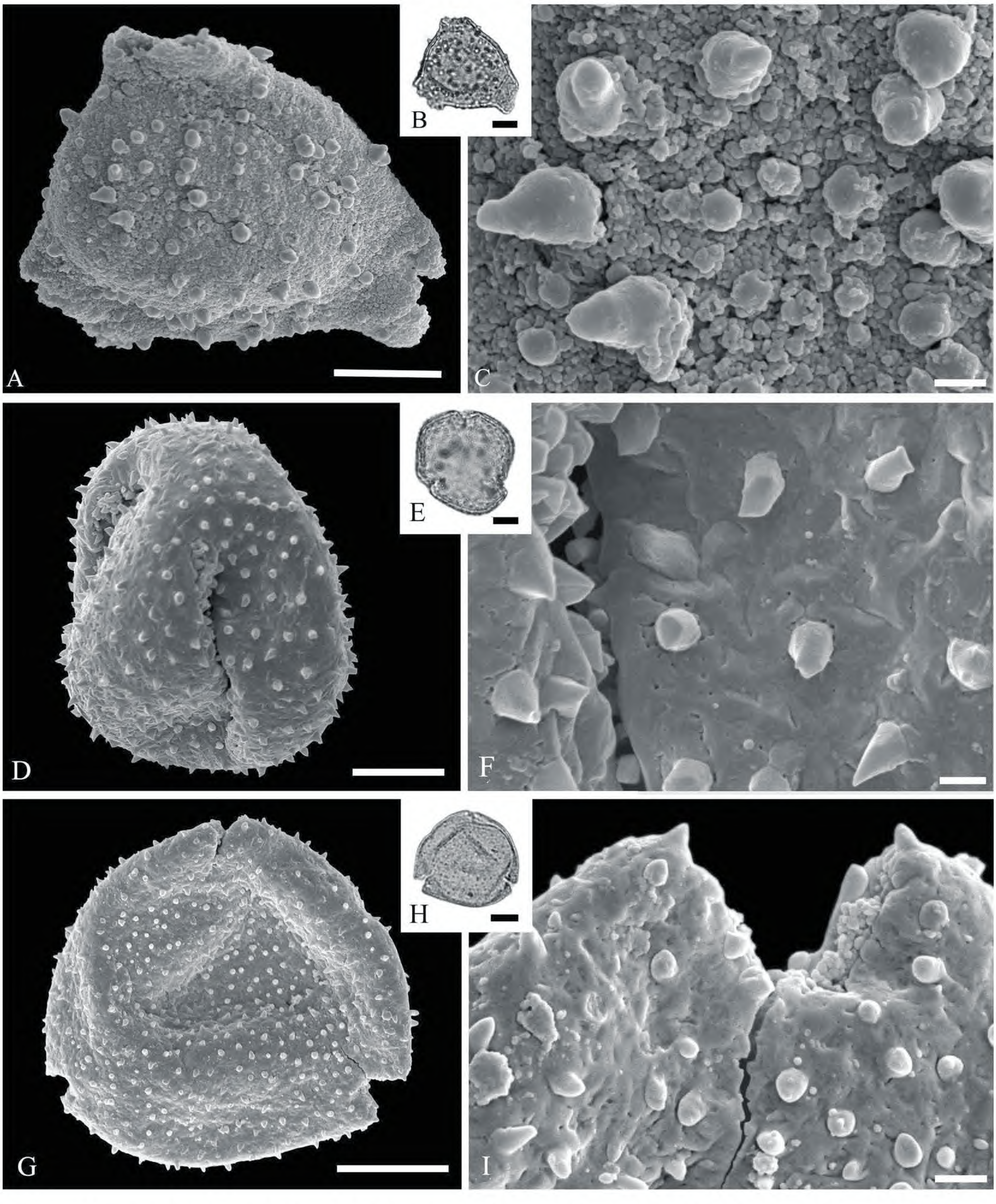


Figure 21. Caprifoliaceae pollen from the Middle Eocene of Hareøen, Greenland (A–C); Profen, central Germany (D–F); and Late Eocene of Florissant, Colorado (G–I). A–C. *Diervilla* Mill. sp. —A. SEM, polar view. —B. LM of the same grain, polar view. —C. Close-up showing microverrucate and echinate sculpturing in polar area. D–F. Caprifoliaceae gen. et spec. indet. —D. SEM, equatorial view. —E. LM of same grain, polar view. —F. Close-up showing echinate, perforate, and fossulate sculpturing in mesocolpium. G–I. *Lonicera* L. sp. —G. SEM, polar view. —H. LM of same grain, polar view. —I. Close-up showing echinate to microechinate perforate tectum. Scale bars: A, B, D, E, G, H = 10 μm; C, F, I = 1 μm.

examples of *Viburnum* pollen are the clade Lentago, from the Middle Miocene of Iceland (Denk et al., 2011: pl. 4.9). Another type of *Viburnum* pollen we document from the Eocene of the Princeton Chert of British Columbia (Fig. 20A–C) shows the high reticulum with numerous free-standing columellae in the luminae, typical of, e.g., *Solenotinus* (DC.) Spach and *Tinus* Mill. clades and in *V. clemensae* J. Kern (M. J. Donoghue, pers. comm., 2014).

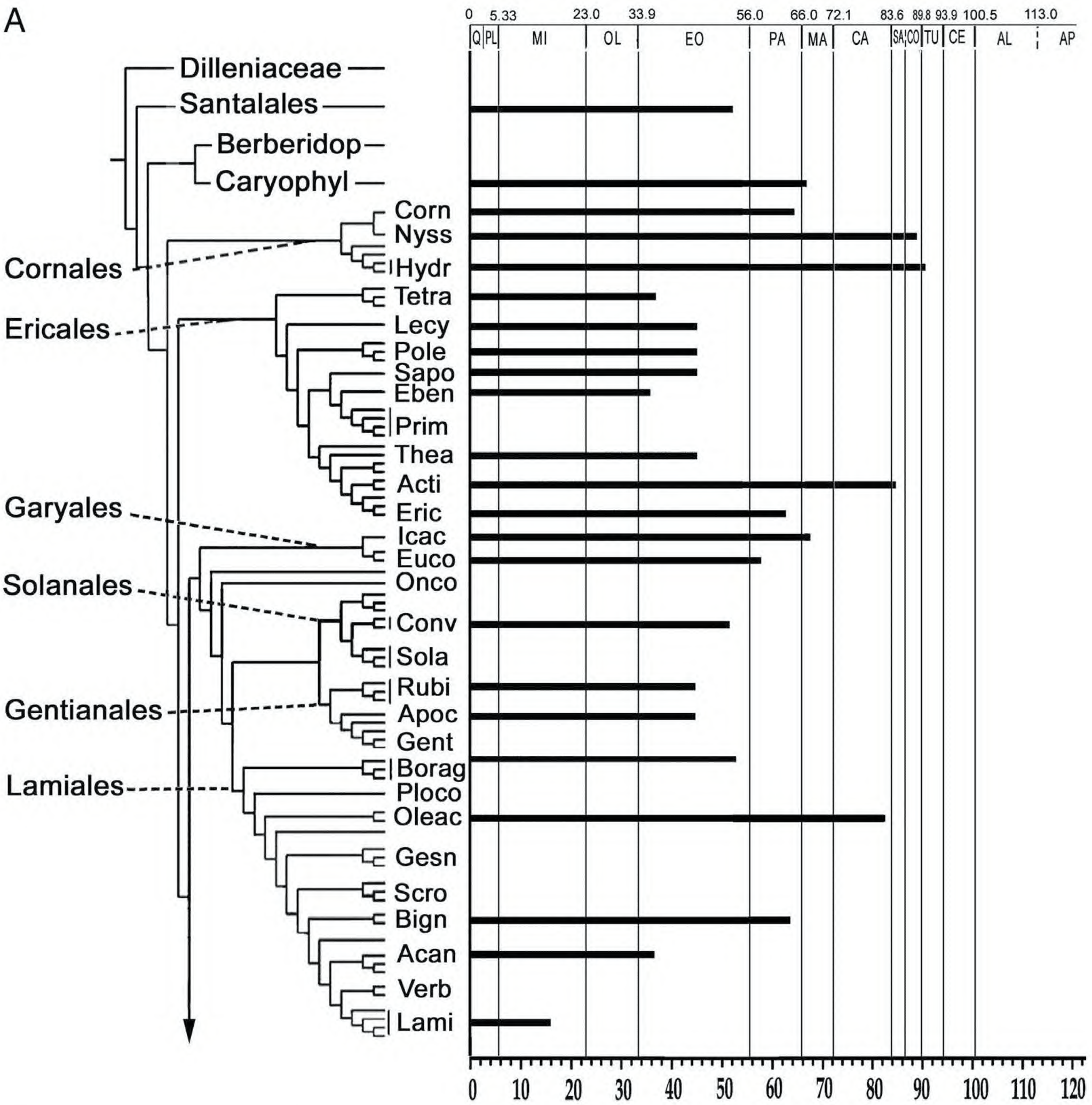


Figure 22. Stratophylogram, plotting some accepted stratigraphic occurrences of Asterid fossils against the phylogenetic topology provided by Soltis et al. (2011), using the age correlations of Gradstein et al. (2012). —A. Cornales, Ericales, and Lamiids. —B. Campanulids. Abbreviations: Q = Quaternary; PL = Pliocene; MI = Miocene; OL = Oligocene; EO = Eocene; PA = Paleocene; MA = Maastrichtian; CA = Campanian; SA = Santonian; CO = Coniacian; Tu = Turonian; CE = Cenomanian; AL =Albian; AP = Aptian. Plant family names, abbreviated to the first four or five characters, are spelled out fully in the phylogram of Figure 1.

Many of the published megafossil reports of *Viburnum* have not held up to close scrutiny. The Paleocene leaf and fruit records formerly assigned to species of *Viburnum* have all been discredited, with reassignments to Cornales as *Beringiaphyllum* (Manchester et al., 1999), *Davidia* (Manchester, 2002), and *Browniea* (Manchester & Hickey, 2007) and to Cannabaceae with the transfer of *V. asperum* Newberry to *Celtis* L. (Manchester et al., 2002).

Dipsacales are also known from winged fruits of Linnaeoideae (Caprifoliaceae) from the Late Eocene (~36 Ma), representing the extant genus *Dipelta* in the Late Eocene of southern England (Reid & Chandler, 1926) and Mississippi (Manchester et al., 2009a) and the extinct genus *Diplodipelta* Manchester & Donoghue 1995. Several pollen types present among extant Dipsacales are distinctive (Donoghue, 1985) and readily recognizable in the fossil record. Here, we provide examples of the distinctive echinate pollen of Linnaeoideae from the Middle Eocene of Greenland (Fig. 21A–C) and Germany (Fig. 21D–F) and *Lonicera* L. from the Late Eocene of Colorado, U.S.A. (Fig. 21G–I).

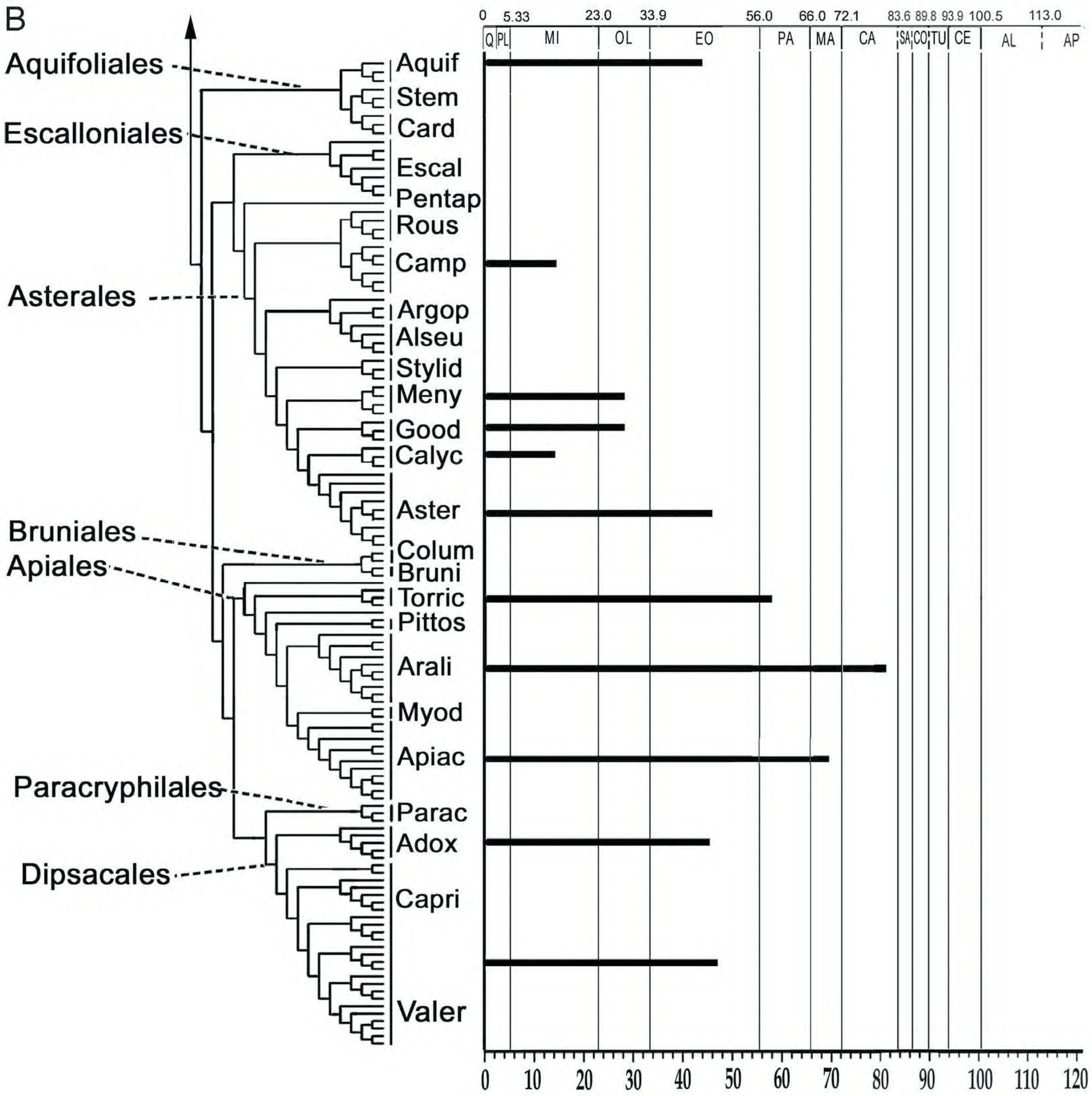


Figure 22. Continued.

Conclusions

Although many gaps remain, particularly for the record of dominantly herbaceous families, the increased recovery of new fossils over recent decades, combined with the development of new methods for more easily extracting more morphological and anatomical data from fossils, gives optimism that the paleobotanical record will provide an increasingly robust framework for understanding the timing and pattern of asterid evolution.

Figure 22 is an attempt to show, in a simplistic way, the distribution of the fossil data that have been reviewed here in relation to the phylogenetic topology recently inferred from a 17-gene analysis of the angiosperms with good representative sampling across the Asterids (Soltis et al., 2011). The earliest unequivocal asterid fossils are members of Cornales extending back to the Turonian (~89 Ma), followed by Ericales to the Santonian (~84 Ma), the Lamiales first known from the Early Campanian (~83 based on Oleaceae pollen), and Apiales also to the Campanian (Araliaceae pollen). Others, like Dipsacales, are not yet confirmed from pre-Cenozoic sediments. The densest records are for those of Cornales and Ericales, which happen to be "lower" in the phylogeny. Dominantly herbaceous taxa have a poorer and/or younger record. Nevertheless, Asteraceae were already widespread in the Eocene based on pollen records.

Modern genera of some clades become recognizable, in the case of Cornales, Ericales, and Dipsacales, by the Early Eocene (in the Late Cretaceous for *Davidia* and *Nyssa*), but at the same time, extinct genera in these clades are obvious. The fossil record for many lamiid and campanulid families remains poorly known, but is improving with increased attention to high-resolution investigations of dispersed pollen as well as the occasional recovery of flowers and fruits. Targeted studies of fossil pollen (including both SEM and LM) as well as flowers, fruits, and seeds from regions of particular interest will provide improved resolution of the timing of diversification within this group of angiosperms that has become a dominant component of modern vegetation.

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TAXONOMIC REVISION OF THE MOSS GENUS *LEPIDOPILIDIUM* (PILOTRICHACEAE)[1]

John J. Atwood[2]

Abstract

Lepidopilidium (Müll. Hal.) Broth. (Pilotrichaceae) consists of mainly epiphytic and epiphyllous, pleurocarpous mosses distributed in the tropical, subtropical, and south temperate regions of Central America, South America, Africa, southern India, and Sri Lanka. The genus is characterized by a moderately to strongly complanate habit; the presence of a moderately to well-developed stem hyalodermis; dimorphic dorsal, ventral, and lateral leaves; prominent double costae; smooth, hexagonal, rhomboidal, or fusiform leaf cells; smooth or papillose setae; collenchymatous exothecial cells; 2-celled stomata; massive annuli; a hookeriaceous-type peristome; and sparsely pilose or glabrous mitrate calyptrae. Six species are taxonomically accepted: *L. brevisetum* (Hampe) Broth., *L. devexum* (Mitt.) Broth., *L. divaricatum* (Dozy & Molk.) Broth., *L. furcatum* (Thwaites & Mitt.) Broth., *L. isleanum* (Besch.) Broth., and *L. nitens* (Hornsch.) Broth., with 19 names newly synonymized. *Lepidopilum chenagonii* Renauld & Cardot is transferred to *Thamniopsis* (Mitt.) M. Fleisch., as *T. chenagonii* (Renauld & Cardot) J. J. Atwood. *Lepidopilidium crispifolium* W. R. Buck & Wigginton and *Hookeria pallidifolia* Mitt. are placed in *Hookeriopsis* (Besch.) A. Jaeger s. str., as *H. crispifolia* (W. R. Buck & Wigginton) J. J. Atwood and *H. pallidifolia* (Mitt.) Geh. & Herzog, respectively. *Lepidopilum plebejum* (Müll. Hal.) Sehnem is treated as a new synonym of *Lepidopilum pallidonitens* (Müll. Hal.) Paris. Typification is designated for the unranked Lamprophyllum group of *Hookeria* Sm. by *Hookeria nitens* Hornsch. [≡ *Lepidopilidium nitens*] and for *Lepidopilum* sect. *Plagiotheciella* Besch. by *Lepidopilum isleanum* Besch. [≡ *Lepidopilidium isleanum*]. Typifications are designated for *Crossomitrium portoricense* Müll. Hal. [≡ *Lepidopilidium portoricense* (Müll. Hal.) H. A. Crum & Steere; = *L. nitens*], *Hookeria aureopurpurea* Geh. & Hampe [≡ *Lepidopilidium aureopurpureum* (Geh. & Hampe) Broth.; = *L. nitens*], *Hookeria aureopurpurea* Müll. Hal. [≡ *Lepidopilidium lamprophylloides* (Paris) Broth.; = *L. brevisetum*], *Hookeria breviseta* Hampe [≡ *Lepidopilidium brevisetum*], *Hookeria divaricata* Dozy & Molk. [≡ *Lepidopilidium divaricatum*], *Hookeria entodontella* Müll. Hal. ex Broth. [≡ *Lepidopilidium entodontella* (Müll. Hal. ex Broth.) Broth.; = *L. nitens*], *Hookeria gracilifrons* Müll. Hal. [≡ *Lepidopilidium gracilifrons* (Müll. Hal.) Broth.; = *L. nitens*], *Hookeria longicuspis* Müll. Hal. [≡ *Lepidopilidium longicuspis* (Müll. Hal.) Broth.; = *L. nitens*], *Hookeria nitens* Hornsch. [≡ *Lepidopilidium nitens*], *Hookeria subnitens* Geh. & Hampe [≡ *Lepidopilidium subnitens* (Geh. & Hampe) Broth.; = *L. nitens*], *Hookeria subnitens* Geh. & Hampe var. *latior* Geh. & Hampe [= *Lepidopilidium nitens*], *Hookeria tenuiseta* Müll. Hal. [≡ *Lepidopilidium tenuisetum* (Müll. Hal.) Broth.; = *L. nitens*], *Hookeria wainioi* Broth. [≡ *Lepidopilidium wainioi* (Broth.) Broth.; = *L. nitens*], *Lepidopilum corbieri* Renauld & Cardot [≡ *Lepidopilidium corbieri* (Renauld & Cardot) Cardot; = *L. divaricatum*], *Lepidopilum devexum* Mitt. [≡ *Lepidopilidium devexum*], *Lepidopilum flexuosum* Besch. [≡ *Lepidopilidium flexuosum* (Besch.) Broth. ex Paris; = *L. isleanum*], *Lepidopilum fruticola* Müll. Hal. [≡ *Lepidopilidium fruticola* (Müll. Hal.) Broth.; = *L. brevisetum*], *Lepidopilum furcatum* Thwaites & Mitt. [≡ *Lepidopilidium furcatum*], *Lepidopilum hanningtonii* Mitt. [≡ *Lepidopilidium hanningtonii* (Mitt.) Broth.; = *L. furcatum*], *Lepidopilum isleanum* [≡ *Lepidopilidium isleanum*], *Lepidopilum laevisetum* Hampe [≡ *Lepidopilidium laevisetum* (Hampe) Broth.; = *L. nitens*], *Lepidopilum subdevexum* Broth. [≡ *Lepidopilidium subdevexum* (Broth.) Broth.; = *L. devexum*], *Lepidopilum subdivaricatum* Renauld & Cardot [≡ *Lepidopilidium subdivaricatum* (Renauld & Cardot) Broth.; = *L. divaricatum*], *Lepidopilum subrevolutum* Renauld & Cardot [≡ *Lepidopilidium subrevolutum* (Renauld & Cardot) Cardot; = *L. isleanum*], *Lepidopilidium attenuatum* Thér. [= *L. furcatum*], *Lepidopilidium brevisetum* (Hampe) Broth. var. *purpurascens* Broth. [= *L. brevisetum*], *Lepidopilidium parvulum* Schimp. ex Cardot [= *L. isleanum*], *Lepidopilidium theriotii* Naveau [= *L. furcatum*], *Lepidopilidium subdevexum* Broth. (Broth.) var. *intermedium* P. de la Varde [= *L. devexum*], and *Lepidopilidium synoicum* Herzog [= *L. divaricatum*].

Key words: Africa, Central America, *Hookeriopsis*, India, *Lepidopilidium*, *Lepidopilum*, Pilotrichaceae, South America, Sri Lanka, *Thamniopsis*.

Within the Hookeriales there are several generic pairs that are gametophytically similar to each other but have dissimilar sporophytes. Such pairings include *Crosbya* Vitt and *Daltonia* Hook. & Taylor, *Distichophyllum* Dozy & Molk. and *Leskeodon* Broth., *Stenodesmus* (Mitt.) A. Jaeger and *Stenodictyon* (Mitt.) A. Jaeger, and *Lepidopilidium* (Müll. Hal.) Broth. and *Lepidopilum* (Brid.) Brid. Gametophytic similarities between *Lepidopilidium* and *Lepidopilum* species are particularly seen in the scabrisetum group based on *Lepidopilum scabrisetum* (Schwägr.) Steere. Of the 40 validly published names in *Lepidopilidium*, 18 were

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at one time placed in *Lepidopilum*. *Lepidopilidium* and the *Lepidopilum* species in the scabrisetum group are characterized by a moderately to strongly complanate habit; the presence of a stem hyalodermis; 2-celled axillary hairs; rhizoids clustered on the ventral surface of the stem or abaxial to the leaf insertion; dimorphic dorsal, ventral, and lateral leaves; prominent double costae; and smooth, hexagonal, rhomboidal, or fusiform leaf cells. Despite these gametophytic similarities, the sporophytes of the two genera are mostly at extremes with each other. *Lepidopilidium* has smooth to papillose setae and sparsely pilose or glabrous calyptrae, whereas *Lepidopilum* has papillose to scabrous setae and pilose to rarely glabrous calyptrae. However, the best means of distinguishing *Lepidopilidium* from *Lepidopilum* is by their different peristome types. *Lepidopilidium* has a hookeriaceous-type peristome that is composed of dark red, narrowly triangular exostome teeth that are furrowed, thickened, proximally cross-striate, and distally papillose on the outer (dorsal) surface. The endostome consists of a high basal membrane and finely papillose segments. By contrast, *Lepidopilum* has a daltoniaceous-type peristome that consists of pale, linear to very narrowly triangular exostome teeth that lack median furrows and are thin and papillose on the outer (dorsal) surface throughout. The endostome is composed of papillose segments above a high or low basal membrane. These two contrasting peristome types further differ in their hygroscopic movement. The exostome teeth in the hookeriaceous peristome type of *Lepidopilidium* are incurved when hydrated and reflexed when dehydrated, whereas in the daltoniaceous peristome type of *Lepidopilum* they are incurved when dehydrated and reflexed when hydrated (Whittemore & Allen, 1989).

The oldest known name in *Lepidopilidium* (Pilotrichaceae) is *Hookeria nitens* Hornsch. (Hornschuch, 1840). This species and *H. glaziovii* Hampe [= *Thamniopsis langsdorffii* (Hook.) W. R. Buck] were considered by Hampe (1874) within the unranked Lamprophyllum group of *Hookeria* Sm., which included species that were superficially similar to *Lepidopilum* but differed in having smooth setae ornamentation and a hookeriaceous-type peristome. Additional species were subsequently added to the group by Hampe (1878), Geheeb and Hampe (1881), and Müller (1900), who in addition established another group in *Hookeria*, parenthetically cited as "Lepidopilidium." Müller's intentions for the Lepidopilidium group are unclear since he did not formally designate a rank. In the same publication (1900: 235–236), however, he cites "Pseudepheme-rum" in a similar manner within the new species *Ephemerum perexiguum* Müll. Hal. His intention of designating a new section within *Ephemerum* Hampe is found in the diagnoses. Following the provision of Article 37.5 of the International Code of Botanical Nomenclature (McNeill et al., 2012), all parenthetically cited names in this paper should be treated similarly, in this case as sections. *Hookeria* sect. *Lepidopilidium* Müll. Hal. was noted as superficially similar to *Lepidopilum*, but it differed in having a hookeriaceous- versus daltoniaceous-type peristome. Three species newly described by Müller (1900) were placed in this section: *Hookeria* (*Lepidopilidium*) *tenuiseta* Müll. Hal., *Hookeria* (*Lepidopilidium*) *longicuspis* Müll. Hal., and *Hookeria* (*Lepidopilidium*) *rupestris* Müll. Hal., the first of which was later designated by Welch (1969) as the lectotype of *Lepidopilidium*.

Brotherus (1907) elevated *Hookeria* sect. *Lepidopilidium* to generic rank and significantly expanded it by including species from the unranked Lamprophyllum group of *Hookeria* and *Lepidopilum*. The genus then encompassed 24 species that were separated by variation in lateral leaves, sexuality, setae length and ornamentation, capsule shape and orientation, and spore size. Regional treatments of *Lepidopilidium*, as well as revisions of related genera, have since resulted in the description of seven more taxa (Renauld & Cardot, 1915; Herzog, 1916; Brotherus, 1924; Thériot, 1930; Potier de la Varde, 1939; Dixon & Thériot, 1942; Wigginton & Buck, 2013), the transfer of 10 taxa to *Lepidopilidium* (Paris, 1909; Renauld & Cardot, 1915; Crum & Steere, 1956; Sehnem, 1979; Buck & Ireland, 1989; Vaz-Imbassahy & Costa, 2009), one transfer out of *Lepidopilidium* to *Lepidopilum* (Sehnem, 1979; Vaz & Costa, 2006; Vaz-Imbassahy & Costa, 2008), and the placement of eight names into synonymy within *Lepidopilidium* (Brotherus, 1924; Buck, 1998; Churchill et al., 2000; Vaz & Costa, 2006; Vaz-Imbassahy & Costa, 2008; Allen, 2010). Among the now 40 validly published names in *Lepidopilidium*, 29 species are currently recognized, along with two varieties.

Lepidopilidium has never been the subject of a worldwide revision, and the delimitation of taxa as well as their distributions are not sufficiently resolved (Buck, 1998; Gradstein et al., 2001; Allen, 2010; Costa et al., 2011; Wigginton & Buck, 2013). Additionally, *Lepidopilidium* is reported to be paraphyletic in its present circumscription (Buck et al., 2005), with one *Lepidopilidium* accession nested within a clade with *Lepidopilum* and *Stenodictyon wrightii* (Sull. & Lesq.) Crosby, while a second is

nested in a clade with *Thamniopsis* (Mitt.) M. Fleisch. *Lepidopilidium*, as circumscribed here, is considered to be monophyletic. In the phylogeny by Buck et al. (2005), the alignment of the *Lepidopilidium laevisetum* accession (*Schäfer-Verwimp 9237*, NY) with *Thamniopsis* is in agreement with their similar morphology. All members of this clade share a hookeriaceous-type peristome. On the other hand, the alignment of the *Lepidopilidium portoricense* accession (*Buck 37825*, NY) with *Lepidopilum* and *Stenodictyon* couples hookeriaceous and daltoniaceous peristome types together in a clade. A re-examination of the *Lepidopilidium portoricense* specimen found it to be a misdetermined specimen of *Lepidopilum scabrisetum*. As previously noted, sporophyte characters provide the best means for separating *Lepidopilidium* from *Lepidopilum*, although sporophytes are lacking in this specimen. Utilizing gametophyte characters is much more difficult as it requires familiarity with the species in *Lepidopilidium* as well as the scabrisetum group of *Lepidopilum*. Welch (1976) observed that the median leaf cells in *Lepidopilidium* are usually wider than those of *Lepidopilum* and have a length:width ratio of 10:1. Conversely, the median leaf cells of *Lepidopilum* are usually longer and narrower, with a ratio of 22:1. In addition, the lateral leaves of *Lepidopilum scabrisetum* tend to be more sigmoid in shape than those of *Lepidopilidium*. Allen (2010) noted that *Lepidopilidium* sometimes has narrowly recurved upper leaf margins, whereas the upper leaf margins of species in the scabrisetum group in *Lepidopilum* are plane. Lastly, most Neotropical *Lepidopilidium* are dioicous, whereas most Neotropical specimens of *Lepidopilum scabrisetum* are autoicous, although dioicous forms exist (Churchill, 1988). By replacing the *Lepidopilidium portoricense* accession with *Lepidopilum scabrisetum*, a more morphologically related clade is obtained, now composed of two *Lepidopilum scabrisetum* accessions, *L. polytrichoides* (Hedw.) Brid. and *Stenodictyon wrightii*. Since *S. wrightii* has a hookeriaceous-type peristome, it remains an oddity within this otherwise daltoniaceous-type peristome clade. Also peculiar is that another *Stenodictyon* species included in the phylogeny appears to be unrelated. A possible explanation for this incongruity is that the *S. wrightii* accession may also be based on a misdetermination. The corresponding specimen was not re-examined in this study. However, according to Allen (2010), the former concept of *S. wrightii*, presented in a review of the genus (Allen et al., 1986), confuses non-sporophyte material with several other species. Among these species are *Lepidopilum longifolium* Hampe and *Stenodesmus tenuicuspis* (Mitt.) A. Jaeger, both of which have a daltoniaceous-type peristome.

Lepidopilidium is currently recognized to contain the following six species: *L. brevisetum* (Hampe) Broth., *L. devexum* (Mitt.) Broth., *L. divaricatum* (Dozy & Molk.) Broth., *L. furcatum* (Thwaites & Mitt.) Broth., *L. isleanum* (Besch.) Broth., and *L. nitens* (Hornsch.) Broth. In addition, 19 names are newly synonymized, five names are transferred to other genera, and five names remain insufficiently known because their type specimens could not be located.

Materials and Methods

This taxonomic revision is based on approximately 40 type and 600 non-typic specimens from 14 herbaria (B, BM, BR, FH, G, H, JE, L, M, MO, NY, PC, S, US). Although all specimens were examined and annotated using standard practices, only those with complete and adequate material for sampling were used in the descriptions and illustrations. All measurements were made using rehydrated material mounted in Hoyer's solution (Anderson, 1954). Leaves selected for measurements were removed from the middle of the stem. Lamina length was measured from the leaf tip to the base, and lamina width was measured at the widest point. Cellular measurements include the cell wall. Measurements of the capsule urn exclude the operculum. The peristome was measured from the tip of the exostome teeth or endostome segment to the capsule mouth. Spore mounts were made from mature capsules, in which the operculum was removed after rehydration. The terminology for bryological characters follows Magill (1990). The taxonomic nomenclature includes the complete type citation from the original protologue. Species are defined using a taxonomic species concept that delimits groups according to their overall similarities. Distribution and habitat data are based on herbarium specimen labels. All specimens examined in this study are cited, although some details regarding their locality have been shortened or omitted for brevity.

Taxonomic Treatment

Lepidopilidium (Müll. Hal.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 942. 1907. *Hookeria* Sm. sect. *Lepidopilidium* Müll. Hal., Hedwigia 39: 272. 1900. TYPE: *Hookeria tenuiseta* Müll. Hal. (lectotype, designated by Welch, 1969: 123).

Hookeria [unranked] *Lamprophyllum* Hampe, in Warm., Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn ser.

3, 6: 158. 1874. TYPE: *Hookeria nitens* Hornsch. (lectotype, designated here).

Lepidopilum sect. *Plagiotheciella* Besch., Ann. Sci. Nat. Bot., sér. 6, 10: 279. 1880. TYPE: *Lepidopilum isleanum* Besch. (lectotype, designated here).

Plants dull or glossy, yellow, golden, green, or brown, often tinted red or purple, in lax to dense mats, occasionally tufts; stems prostrate, to 7 cm, spreading or erect, unbranched or irregularly branched with numerous short branches, moderately to strongly complanate-foliate; in cross-section with a moderately to well-developed hyalodermis, 2 to 4 rows of small, yellowish brown, thick-walled, subepidermal cells and large, hyaline, thin-walled, cortical cells; central strand absent; paraphyllia and pseudoparaphyllia absent; axillary hairs 2-celled, basal cell hyaline or brownish hyaline, short-rectangular, upper cell hyaline, oblong; rhizoids reddish brown, smooth, irregularly branched, arising from clusters of initials on ventral surface of primary stems, abaxial to leaf insertion and at base of costae. Leaves arranged in 8 rows, evenly or densely spaced, weakly to strongly dimorphic, flat or weakly concave, shriveled, contorted, or flexuous, appressed, erect, erect-spreading, or wide-spreading when dry. Dorsal and ventral leaves symmetric or weakly asymmetric, elliptical, ovate, oblong-ovate, or oblong-lanceolate, abruptly acute to short-acuminate. Lateral leaves weakly symmetric to strongly asymmetric, elliptical, ovate, oblong-ovate, oblong-lanceolate, or lanceolate, straight or falcate, abruptly acute, broadly to narrowly, short- or long-acuminate, sometimes slenderly acuminate; margins serrulate, serrate, or sharply serrate above, a few teeth occasionally inflated or bifurcate, serrulate to entire below, plane or more often narrowly recurved, sometimes broadly infolded on one side at base, straight to rounded to the insertion, elimbate or with 1 or 2 rows of elongate, narrow cells; costae double, prominently extending to about midleaf, parallel or diverging throughout, uneven, smooth or weakly serrate-toothed at back, not projecting or strongly projecting at tips; laminal cells smooth, thin- or thick-walled, sometimes porose, apical and median cells hexagonal, rhomboidal, or fusiform, becoming oblong-rectangular below; alar cells not differentiated; a single row of rounded, bulging cells along leaf insertion. Asexual reproduction by hyaline or reddish brown, smooth, filamentous, multicellular, uniseriate gemmae, arising from clusters of initials abaxial to leaf insertion and at base of costae. Plants autoicous, synoicous, or dioicous. Setae short to elongate, yellow to red, smooth throughout, smooth below and lightly or densely papillose above, or papillose throughout. Capsules red-brown, erect, suberect, or inclined, obloid to obloid-cylindrical, neck well-developed; exothecial cells subrectangular, collenchymatous; stomata on neck, 2-celled, phaneroporic; opercula short to long conic-rostrate; annuli of 3 to 10 rows of fragmenting, quadrate cells, persistent on opercula or capsule mouth; peristome diplolepideous; exostome teeth narrowly triangular, median furrows present, outer (dorsal) surface dark red, proximally cross-striate, distally papillose, inner (ventral) surface yellow, lightly papillose; endostome segments about as long as exostome, pale to yellow, lightly papillose throughout, concave-keeled, narrowly perforate, cilia absent. Spores spherical, yellow, finely papillose. Calyptrae mitrate, pale to yellow or red, smooth to weakly scabrous at tip, lacinate at base, sparsely pilose or glabrous.

Nomenclatural notes. Müller (1900) simultaneously described the species *Hookeria tenuiseta*, *H. longicuspis*, and *H. rupestris* in *Hookeria* sect. *Lepidopilidium*. Although all three species share equal priority, Welch (1969) selected *H. tenuiseta* as the lectotype of the genus without further justification. Her seemingly mechanical method of designating generic types with the first published name follows a pattern used for type selection by her in other genera, e.g., *Amblytropis* (Mitt.) Broth., *Hypnella* (Müll. Hal.) A. Jaeger, and *Hookeriopsis* (Besch.) A. Jaeger. Crosby et al. (1985) found reason to supersede the mechanically selected lectotype in *Hypnella*. In *Lepidopilidium*, however, Welch's type selection is retained due to the short generic description that follows *H. tenuiseta*.

Hampe's (1874) Lamprophyllum group in *Hookeria* was established for *H. nitens* [≡ *Lepidopilidium nitens*] and *H. glaziovii* [= *Thamniopsis langsdorffii*]. *Hookeria nitens* is designated herein as the type of this unranked group.

Lepidopilum sect. *Plagiotheciella* was established by Bescherelle (1880) to include *L. isleanum* and *L. cespitosum* Besch. The type of *L. cespitosum* could not be located, although the taxon appears to better affine within *Thamniopsis*. *Lepidopilum isleanum* is designated herein as the type of this section.

Discussion. *Lepidopilidium* forms mostly lax to dense, moderately to strongly complanate-foliate mats of spreading, prostrate to erect stems, up to 7 cm in length. Occasionally, as in *L. furcatum*, plants sometimes form tufts of erect stems. In cross-section, the stems have a moderately to well-developed hyalodermis; two to four rows of small, yellowish brown, thick-walled subepidermal cells; and large, hyaline, thin-walled, cortical cells. Rhizoid initials are located along the ventral surface of the stem or

clustered abaxial to the leaf insertion and at the base of the costae. The clusters of initials may produce rhizoids, gemmae, or both. The rhizoids are reddish brown, smooth, and irregularly branched. The gemmae are filamentous, hyaline or reddish brown, smooth, uniseriate, and multicellular, and occur abundantly in *L. brevisetum*, *L. furcatum*, *L. isleanum*, and *L. nitens*. Other features of *Lepidopilidium* include 2-celled axillary hairs and the lack of paraphyllia and pseudoparaphyllia. Foliose, short-triangular primordial leaves clustered around the branch buds can be confused for pseudoparaphyllia.

Due to the complanate-foliate habit of the stems, the dorsal and ventral leaves differ from lateral leaves in size, symmetry, and shape. The dorsal and ventral leaves are typically appressed or erect; symmetric or weakly asymmetric; ovate, oblong-ovate, or oblong-lanceolate; and have acute to shortly acuminate apices. Much of the described variation in *Lepidopilidium* is based on lateral leaf characters. The lateral leaves are erect, erect-spreading, or wide-spreading; weakly symmetric to strongly asymmetric; and are interspecially and intraspecially polymorphic in leaf shape and size as well as in cell areolation. Leaves near the branch tips tend to be longer, have more attenuate apices, and have longer and wider cells than leaves lower on the stem. The oldest leaves tend to be shorter, less acuminate, and have broadly rhomboidal cells. Leaf shape varies from elliptical (*L. nitens*) to ovate or oblong-ovate (*L. divaricatum*, *L. isleanum*, and *L. nitens*), ovate-lanceolate (*L. brevisetum* and *L. furcatum*), oblong (*L. brevisetum*, *L. nitens*, *L. devexum*, *L. divaricatum*, *L. furcatum*, *L. isleanum*, and *L. nitens*), oblong-lanceolate (*L. brevisetum*, *L. devexum*, *L. divaricatum*, *L. furcatum*, and *L. nitens*), or lanceolate (*L. furcatum* and *L. nitens*). *Lepidopilidium devexum* has distinctly falcate lateral leaves. The leaf apices are also considerably polymorphic, ranging from acute to short- or long-acuminate. The upper leaf margins are often narrowly recurved, while the lower leaf margins are sometimes broadly infolded at the leaf base. The margins are either elimbate (not bordered) or narrowly bordered, can be entire, serrulate, serrate, or sharply serrate, and have a few swollen or bifurcate teeth (*L. furcatum* and *L. nitens*). The double costae prominently extend to about midleaf and mostly stay parallel, although sometimes they diverge. The costae are smooth, but can be weakly toothed at back, and either do not project at the tips as in *L. devexum* and *L. isleanum* or sometimes project as a short terminal cell as in *L. brevisetum*, *L. divaricatum*, *L. furcatum*, and *L. nitens*.

The sexuality, setae length and ornamentation, capsule shape and orientation, and spore size were emphasized by Brotherus (1907) as taxonomically significant characters in *Lepidopilidium*. The perigonia and perichaetia are produced laterally on the stems and branches, are budlike, and have ovate to lanceolate leaves. Based on sexuality and the distribution of species, *Lepidopilidium* appears to have two distinct lineages. *Lepidopilidium brevisetum* and *L. nitens* are dioicous and occur in the Neotropics, whereas *L. devexum*, *L. furcatum*, and *L. isleanum* are autoicous and occur in the Paleotropics. Only the autoicous species *L. divaricatum* occurs in both tropical regions. Synoicous specimens identified as *L. synoicum* Herzog (= *L. divaricatum*) and *L. theriotii* Naveau (= *L. furcatum*) are morphologically identical to autoicous specimens. The setae vary from short (*L. brevisetum* and *L. furcatum*) to elongate (*L. isleanum* and *L. nitens*) and can be straight, somewhat curved, or flexuous. In addition, the ornamentation varies from smooth throughout (*L. brevisetum*, *L. furcatum*, *L. isleanum*, and *L. nitens*) to smooth below and lightly to densely papillose above (*L. brevisetum*, *L. furcatum*, *L. isleanum*, and *L. nitens*) to densely papillose throughout (*L. devexum* and *L. divaricatum*). Capsule orientation appears to be correlated with setae length. Species with short setae have mostly erect capsules, whereas those with elongate setae have suberect to inclined capsules. This character is somewhat variable depending on the direction that the setae-bearing stems were growing at the time of collection. In some species, such as *L. brevisetum*, *L. devexum*, and *L. furcatum*, both erect and inclined capsules were found on the same plant. Additional characteristics of the capsule as well the opercula, peristome, calyptrae, and spores do not noticeably vary between species.

Key to the Species of *Lepidopilidium*

1a. Lateral leaves broadly ovate, oblong-ovate, or oblong, about 2 to 3 times longer than wide; apices acute to abruptly acuminate and ending in a short or long slender point; apical and median leaf cells hexagonal to broadly rhomboidal, sometimes elongate-rhomboidal.
  2a. Gemmae absent; setae short, 5–7.5 mm long ..... 3. *L. divaricatum* (Dozy & Molk.) Broth.
  2b. Gemmae present; setae elongate, 7–15 mm long.
    3a. Lateral leaves rounded to the insertion; plants autoicous; setae flexuous, densely to lightly papillose throughout, sometimes smooth ..... 5. *L. isleanum* (Besch.) Broth.
    3b. Lateral leaves not rounded to the insertion; plants dioicous; setae straight, smooth or sometimes lightly papillose distally ..... 6. *L. nitens* (Hornsch.) Broth.

1b. Lateral leaves oblong, ovate-lanceolate, oblong-lanceolate or lanceolate, often 3 to 6 times longer than wide; apices gradually short- or long-acuminate; upper leaf cells elongate-rhomboidal or fusiform, sometimes short-rhomboidal.
  4a. Dorsal and ventral leaves slightly dimorphic from lateral leaves; lateral leaves 1.7–3 mm long, not falcate, erect, erect-spreading, or wide-spreading when dry; plants dioicous.
    5a. Setae short, 1–4 mm long ..... 1. *L. brevisetum* (Hampe) Broth.
    5b. Setae longer, 7–15 mm long ..... 6. *L. nitens* (Hornsch.) Broth.
  4b. Dorsal and ventral leaves strongly dimorphic from lateral leaves; lateral leaves 2.6–4 mm long, falcate, spreading to wide-spreading when dry; plants autoicous.
    8a. Setae short, 3–5 mm long, smooth or weakly papillose distally .... 4. *L. furcatum* (Thwaites & Mitt.) Broth.
    8b. Setae longer, 5–7 mm long, papillose throughout ..... 2. *L. devexum* (Mitt.) Broth.

1. Lepidopilidium brevisetum (Hampe) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. Basionym: *Hookeria breviseta* Hampe, Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn ser 3, 10: 266. 1878. *Hookeriopsis breviseta* (Hampe) A. Jaeger, Ber. Thatigk. St. Gallischen Naturwiss. Ges. 1877–1878: 510. 1879. TYPE CITATION: [Brazil.] Inter muscos *Glaziovii* pauca specimina carpi. TYPE: [Brazil.] Rio de Janeiro, *A. Glaziou s.n.* (lectotype, designated here, BM!). Figure 1.

Hookeria aureopurpurea Müll. Hal., Hedwigia 39: 278. 1900, nom. illeg. superfl., as "aureo-purpurea," non *Hookeria aureo-purpurea* Geh. & Hampe, Flora 64: 409. 1881. *Lepidopilum aureopurpureum* Müll. Hal., in Ule, Hedwigia 38(Beibl.): 58. 1899, nom. inval. [no description]. Replacement name: *Hookeria lamprophylloides* Paris, Index Bryol. (ed. 2) 2: 336. 1904. *Lepidopilidium lamprophylloides* (Paris) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 687. 1907. TYPE CITATION: Brasilia, Sa. Catharina, Pedras Grandes ad flumen Tubarão superius, in ramulis trunculorum sylvestrium, Aug. 1890, *E. Ule*, Coll. No. *601*; in iisdem locis Junio 1891 c. fr.: idem No. *1183*. TYPE: [Brazil.] Santa Catarina: Tubarão, Aug. 1890, *E. Ule s.n.* (lectotype, designated here, S!; isolectotypes, B!, BR!, FH!, G!, JE!, M!, MO!, PC!, S!, US!).

Lepidopilum fruticola Müll. Hal., Hedwigia 39: 270. 1900, as "fruticolum." *Lepidopilum fruticolum* Müll. Hal., in Ule, Hedwigia 38(Beibl.): 58. 1899, nom. inval. [lacks description]. *Lepidopilidium fruticola* (Müll. Hal.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. *Lepidopilidium fructicosum* (Müll. Hal.) Broth. ex Paris, Coll. Nom. Broth. 17. 1909, orth. var. TYPE CITATION: Brasilia, Sa. Catharina, Serra Geral, in monte Capivare arboretum in declivibus, Januario 1891, *E. Ule*, Coll. No. *1184*; Tubarão, in fruticibus paucis sylvestribus, Julio 1889, idem, Coll. No. *771*, et ad truncos arborum sylvestrium, Aug. 1890, idem, Coll. No. *959*. TYPE: [Brazil.] S. Catarina, Tubarão, "auf Stauden und Gesträuch in eines Waldbache," July 1889, [*Ule*] *771* (lectotype, designated here, H!).

Lepidopilidium brevisetum var. *purpurascens* Broth., in Schiffner (ed.), Ergebn. Bot. Exped. Südbras., Musci 1901, 2: 328. 1924, syn. nov. TYPE CITATION: [Brazil.] São Paulo. In silvis prope Apiahy; ad arbores; ca. 1100 m.s.m. [*Schiffner*] (*1882*). Prope Raiz da Serra; ad arbores in silva; 20–50 m.s.m. [*Schiffner*] (*711*). TYPE: [Brazil.] São Paulo: "In silvis prope Apiahy; ad arbores," ca. 1100 m.s.m., 24 Aug. 1901, *V. Schiffner 1882* (lectotype, designated here, BM!; isolectotype, S!).

Plants glossy, green to golden-brown, sometimes tinted red, in lax mats; stems prostrate, to 4 cm, irregularly and frequently branched, strongly complanate-foliate. Leaves evenly spaced, not or slightly dimorphic, rigid to somewhat flexuous when dry; dorsal and ventral leaves appressed to erect-spreading, symmetric or weakly asymmetric, ovate to oblong, 1.9–2.2 × 0.6–0.8 mm, acute to broadly acuminate; lateral leaves erect to erect-spreading, symmetric or weakly asymmetric, rarely strongly asymmetric, ovate-lanceolate, oblong, or oblong-lanceolate, 1.7–2.5 × 0.5–0.7 mm, gradually acute to broadly or narrowly short-acuminate, occasionally long-acuminate; margins serrate to serrulate above, entire below, plane or narrowly recurved, often broadly folded on one side at base, elimbate; costae extending to midleaf or above, diverging throughout, uneven, smooth, not projecting or weakly projecting at tips; laminal cells smooth, thin-walled; apical and median cells narrowly to broadly fusiform, 35–60 × 10–18 μm, becoming oblong and elongate below; basal cells enlarged, oblong-rectangular. Gemmae present. Plants dioicous. Setae straight or curved, 1–4 mm, yellow to red, smooth or lightly papillose above; capsules erect to suberect, obloid, 1 mm long; opercula short conic-rostrate, 0.8 mm long. Spores 24–30 μm, yellow, finely papillose. Calyptrae 1.7–2 mm long.

Iconography. Brotherus (1907: 244, fig. 605, as *Lepidopilidium lamprophylloides* (Paris) Broth.); Sehnem (1979: 77, tab. IX, fig. b, as *L. brevisetum* (Hampe) Broth.); Vaz and Costa (2006: 979, figs. 1–6, as *L. aureopurpureum* (Geh. & Hampe) Broth.); Vaz-Imbassahy and Costa (2008: 562, figs. 90–95, as *L. aureopurpureum* (Geh. & Hampe) Broth.).

Habitat and distribution. *Lepidopilidium brevisetum* is known from the Brazilian states of Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and São Paulo, where it has been collected on leaves and the branches of trees and treelets in humid forests, often near water at 100–1200 m elevation (Fig. 2A).

Nomenclatural notes. Among the numerous mosses described by Hampe based on the Rio de Janeiro

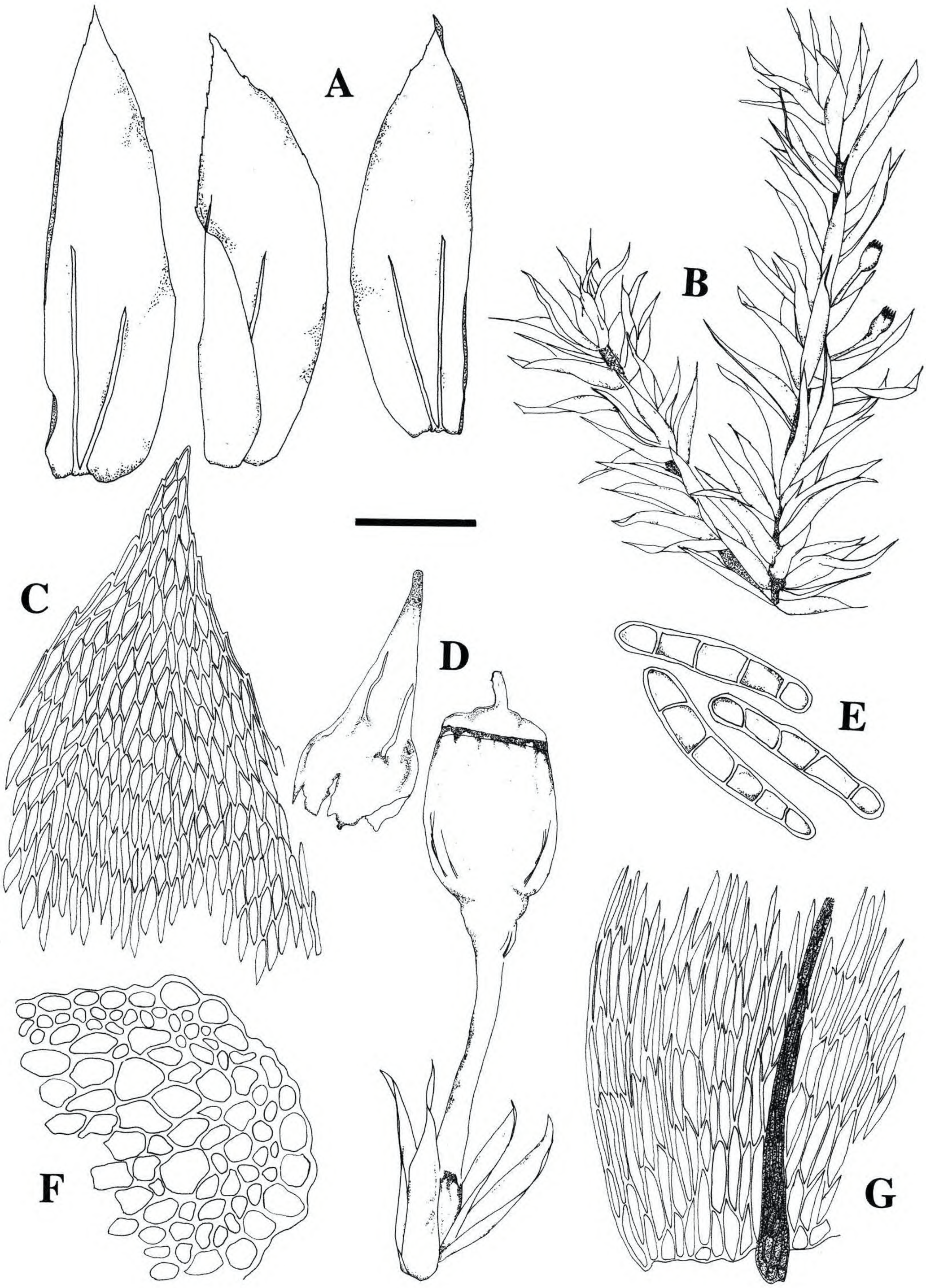


Figure 1. *Lepidopilidium brevisetum* (Hampe) Broth. —A. Lateral leaves. —B. Habit with sporophytes. —C. Lateral leaf apex. —D. Calyptra and sporophyte. —E. Gemmae. —F. Transverse section of stem. —G. Basal laminal cells of lateral leaf. Scale bars: A = 0.6 mm; B = 1.96 mm; C, E, G = 0.13 mm; D = 0.8 mm; F = 0.06 mm. A, C, D, F, G drawn from *Vital & Buck 20584* (MO); B, E drawn from *Bandeira 454* (MO, NY).

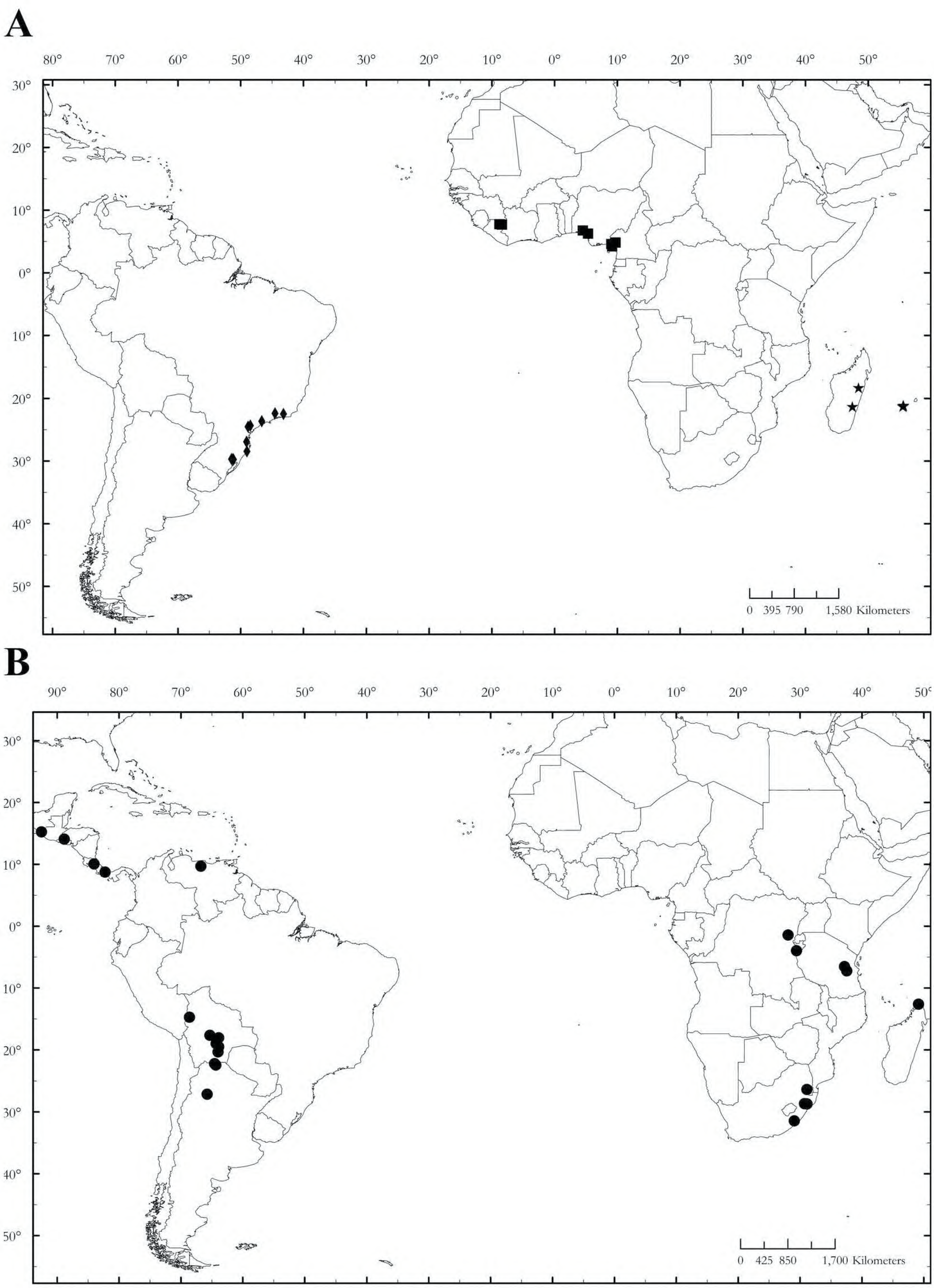


Figure 2. Distributions of *Lepidopilidium* (Müll. Hal.) Broth. species. —A. *Lepidopilidium brevisetum* (Hampe) Broth. (♦), *Lepidopilidium devexum* (Mitt.) Broth. (■), *Lepidopilidium isleanum* (Besch.) Broth. (★). —B. *Lepidopilidium divaricatum* (Dozy & Molk.) Broth. (●).

collections of Glaziou are two species now placed in *Lepidopilidium*. Hampe (1878) described *Hookeria breviseta* [≡ *Lepidopilidium brevisetum*] from a small specimen separated from one of Glaziou's collections. The single specimen lacks a collection number and is in Hampe's herbarium, now deposited in BM. The specimen label as well as the herbarium sheet have Hampe's notes; the specimen appears to be the only one used in the description of the new species. This specimen is selected as the lectotype. The second species, described by Hampe (1879) and now placed in *Lepidopilidium*, is *Lepidopilum laevisetum* [= *Lepidopilidium nitens*]. On some labels the epithet has been misread and reprinted as "brevisetum," although the correlating specimens do not match the type description of *Lepidopilidium brevisetum*.

Hookeria aureopurpurea was described by Müller (1900) on the basis of two syntypic specimens collected by Ule (*601* and *1183*). Among the Ule specimens examined in this study, none had either collection number. Much of the information in Müller's type citation for *Ule 601* matches that on the label of exsiccatal specimen No. *94* (as *Lepidopilum aureopurpureum*) in Ule's (1899) *Bryotheca Brasiliensis*. Specimens in B, BR, FH, G, M, MO, PC, S, and US have "Sa. Catharina, Tubarão" as well as the abbreviated month and year "Aug. 1890." Despite the absence of Ule's collection number *601*, the exsiccatal duplicates are original material. A particularly large specimen with numerous sporophytes in S is designated as the lectotype.

Müller (1900) described *Lepidopilum fruticola* (as "fruticolum") based on three syntypic specimens collected by Ule. The first of these, *Ule 1184*, was cited by Churchill (1988) as belonging to another species, *Lepidopilum subsubulatum* Geh. & Hampe. Another specimen, *Ule 959*, is better identified as *Lepidopilidium nitens*. Only specimen *Ule 771* consistently matches the type description of *Lepidopilum fruticola* and is therefore selected as the lectotype.

Syntype specimens of *Lepidopilidium brevisetum* var. *purpurascens* were both collected from São Paulo, Brazil, in 1901 by Schiffner. These were known to be deposited in BM and S, but were not located in the describing author's herbarium in H. Both specimens equally match the brief type diagnosis. *Schiffner 1882* is selected as the lectotype because it is larger than *Schiffner 711* and has sporophytes.

Discussion. *Lepidopilidium brevisetum* has ovate-lanceolate, oblong or oblong-lanceolate lateral leaves, mostly broad to narrowly short-acuminate apices, and serrate to serrulate upper leaf margins. The costae extend to midleaf or above, are smooth, or occasionally project at the tips. The apical and median laminal cells range from broadly to narrowly fusiform. The setae are short, and the obloid capsules are mostly erect. The only other *Lepidopilidium* species with similarly short setae and mostly erect capsules is *L. furcatum*. That species, however, occurs in Africa, Madagascar, southern India, and Sri Lanka. *Lepidopilidium brevisetum* further differs from *L. furcatum* in dioicous versus autoicous or synoicous plants, less differentiated dorsal, ventral, and lateral leaves, and shorter (1.7–2.5 mm vs. 2.6–4 mm), more symmetrical leaves, with less sharply toothed upper leaf margins. Superficially, *L. brevisetum* is more likely to be confused with *Lepidopilum* species that have strongly complanate-foliate stems, short setae, and erect capsules, such as *L. brevipes* Mitt., *L. cuspidans* Mitt., and *L. subsubulatum*. Although the discernibly different setae ornamentation and peristome will separate the two genera, sterile and mixed specimens can be particularly difficult to differentiate. Ule's (1899) *Bryotheca Brasiliensis* exsiccatal specimen No. *74* is one such mixture. Specimens in BM, H, NY, and S belong mostly to type material of *Lepidopilum nanothecium* Müll. Hal. [= *Lepidopilum brevipes*], whereas specimens in L and S belong mostly to *Lepidopilum fruticola* [= *Lepidopilidium brevisetum*]. Sterile stems in this mixture can be differentiated by the spacing of the lateral leaves when dry. Dry lateral leaves of *Lepidopilidium brevisetum* are more closely spaced and erect-spreading in comparison to the dry lateral leaves of *Lepidopilum brevipes*, which are distantly spaced and wide-spreading.

Vaz and Costa (2006) and Vaz-Imbassahy and Costa (2008) considered the invalid homonym *Lepidopilum aureopurpureum* [≡ *Lepidopilidium lamprophylloides*] to be a synonym of *Lepidopilidium aureopurpureum* (Geh. & Hampe) Broth. However, the type of *Lepidopilidium lamprophylloides* has short setae and erect capsules, whereas the type of *Lepidopilidium aureopurpureum* (as *Hookeria aureopurpurea*) has elongate setae and suberect to inclined capsules and is a synonym of *L. nitens*. Their taxonomic concept of *Lepidopilidium aureopurpureum* otherwise agrees with the concept of *L. brevisetum* presented here. Conversely, their concept of *L. brevisetum* matches the concept of *L. nitens* presented here.

Lepidopilidium brevisetum var. *purpurascens* was described by Brotherus (1924) for two specimens having dark red–colored leaves. Apart from color, pigmented and non-pigmented leaves are morphologically inseparable, and such mixtures can often be found on the same plant.

Additional specimens examined. BRAZIL. **Rio de Janeiro:** Itatiaia, *Bandeira 454* (MO, NY); Tijuca, *Ule 160* (PC). **Rio Grande do Sul:** São Leopoldo, Rio dos Sinos, *Sehnem 198* (FH, NY). **Santa Catarina:** Pedras Grandes, *Ule 691* (PC). **São Paulo:** Apiahy, Sep. 1880, *Puiggari s.n.* (JE), *53b* (G, MO), *631* (JE, L, S); Blumenau, *Ule* [Bryoth. Brasil.] *74*, pro parte (L, S); Iporanga, Serra Paranapiacaba, Faz. Intervales, Rio do Carmo, *Vital & Buck 20584* (MO, NY, SP); Prope Raiz da Serra, *Schiffner 711* (syntype, *Lepidopilidium brevisetum* var. *purpurascens*, BM!).

2. Lepidopilidium devexum (Mitt.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. Basionym: *Lepidopilum devexum* Mitt., J. Proc. Linn. Soc., Bot. 7: 159. 1863. TYPE CITATION: Cameroons Mtn., alt. 4000 ft., *Mann*. TYPE: Cameroon. Cameroon Mtn., 4000 ft., Feb. 1862, *Mann s.n.* (lectotype, designated here, NY!; isotypes, BM!, MO!). Figure 3.

Lepidopilum subdevexum Broth., Bot. Jahrb. Syst. 24: 260. 1897, syn. nov. *Lepidopilidium subdevexum* (Broth.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: Kamerun. an Baumästen bei Ekundu Etitti (*Dusén*). TYPE: Cameroon. "ad Ekundu Etitti emporium," Aug. 1890, *P. Dusén s.n.* (lectotype, designated here, H!; isotypes, BR!, PC!).

Lepidopilum leiopus Müll. Hal., in Paris, Index Bryol. 723. 1898, nom. inval. [lacks description], syn. nov. TYPE: Cameroon. *Dusén* [Musci Africani in Camerunia] *358* (S!).

Plants glossy, golden-yellow, brown to dark red, in lax mats; stems prostrate, to 6 cm, sparsely branched, strongly complanate-foliate. Leaves evenly spaced, somewhat folded, dimorphic; dorsal and ventral leaves appressed, symmetric to weakly asymmetric, ovate-lanceolate, 2–2.7 × 0.6–0.8 mm, short-acuminate; lateral leaves wide-spreading when dry and wet; asymmetric, oblong to oblong-lanceolate, falcate, 2.7–3.3 × 0.5–0.8 mm, narrowly short- to long-acuminate; margins plane or narrowly recurved, often broadly folded on one side at base, sharply serrate above, becoming serrulate to entire below, obscurely bordered by 1 to 2 rows of narrow, elongate cells, confluent at apices; costae extending to midleaf, diverging throughout; uneven, smooth at back, not projecting to weakly projecting at tips; laminal cells smooth, thin-walled; apical and median cells narrowly rhomboidal to fusiform 50–100 × 16–24 μm, becoming elongate-fusiform below. Gemmae absent. Plants autoicous or synoicous. Setae straight or curved, 5–7 mm, yellow to red, papillose throughout; capsules suberect, inclined, or occasionally erect, obloid, 1.3–1.6 mm long, often constricted below the mouth when dry; opercula conic-rostrate, 0.8 mm long. Spores 25–30 μm, yellow, finely papillose. Calyptrae 1.5–1.8 mm long.

Habitat and distribution. *Lepidopilidium devexum* is known from the mountainous regions of western Africa in Guinea, Nigeria, and Cameroon. The species has been collected on branches, twigs, and leaves of shrubs and trees at 1000–1200 m elevation (Fig. 2A).

Nomenclatural notes. *Lepidopilum devexum* was described by Mitten (1863) from a collection made by Mann on the summit of Mount Cameroon, Cameroon. Duplicates of this specimen are deposited in BM, MO, and NY. The specimen in Mitten's herbarium in NY includes accompanying illustrations and is designated as the lectotype.

Original material of *Lepidopilum subdevexum*, described by Brotherus (1897), is represented in BR, H, and PC. The specimen in Brotherus's herbarium in H is the largest of the three duplicates and is designated as the lectotype.

The name *Lepidopilum leiopus* is based on Dusén's *Musci Africani in Camerunia* No. *358*, which lacked a validating description and was excluded from *Lepidopilum* by Churchill (1988). An annotated specimen in S includes a note by Brotherus comparing *L. leiopus* to *Lepidopilidium subdevexum*, a name now synonymous with *Lepidopilidium devexum*. His disposition is followed here.

Discussion. *Lepidopilidium devexum* has strongly complanate-foliate stems, asymmetric, oblong to oblong-lanceolate lateral leaves, with narrowly short- to long-acuminate apices. The species is similar to *L. divaricatum* in having plants autoicous or synoicous with papillose setae 5–7 mm long and obloid, mostly suberect to inclined capsules. *Lepidopilidium devexum* differs in having distinctly wide-spreading lateral leaves that are longer (2.7–3.3 mm vs. 2.2–2.7 mm) and narrower (0.5–0.8 mm vs. 1–1.3 mm). Although the lateral leaves of *L. devexum* overlap in size with *L. furcatum*, that species can be differentiated by its longer leaf apices and shorter, smooth to distally papillose setae. Brotherus (1897) used the name *L. subdevexum* to describe one of Dusén's specimens from Cameroon that was similar to *L. devexum* but had erect, instead of inclined, capsules. In a later treatment of *Lepidopilidium*, Brotherus (1907) emphasized capsule orientation as well as the curvature of the setae as taxonomically important characters. In comparing the types of *L. devexum* and *L. subdevexum*, the differences in capsule orientation and curvature of the setae are readily noticeable. However, considerable variation has been observed in the examination of additional specimens. Both species have setae that curve under the base of the capsule urn, resulting in a mixture of erect, suberect,

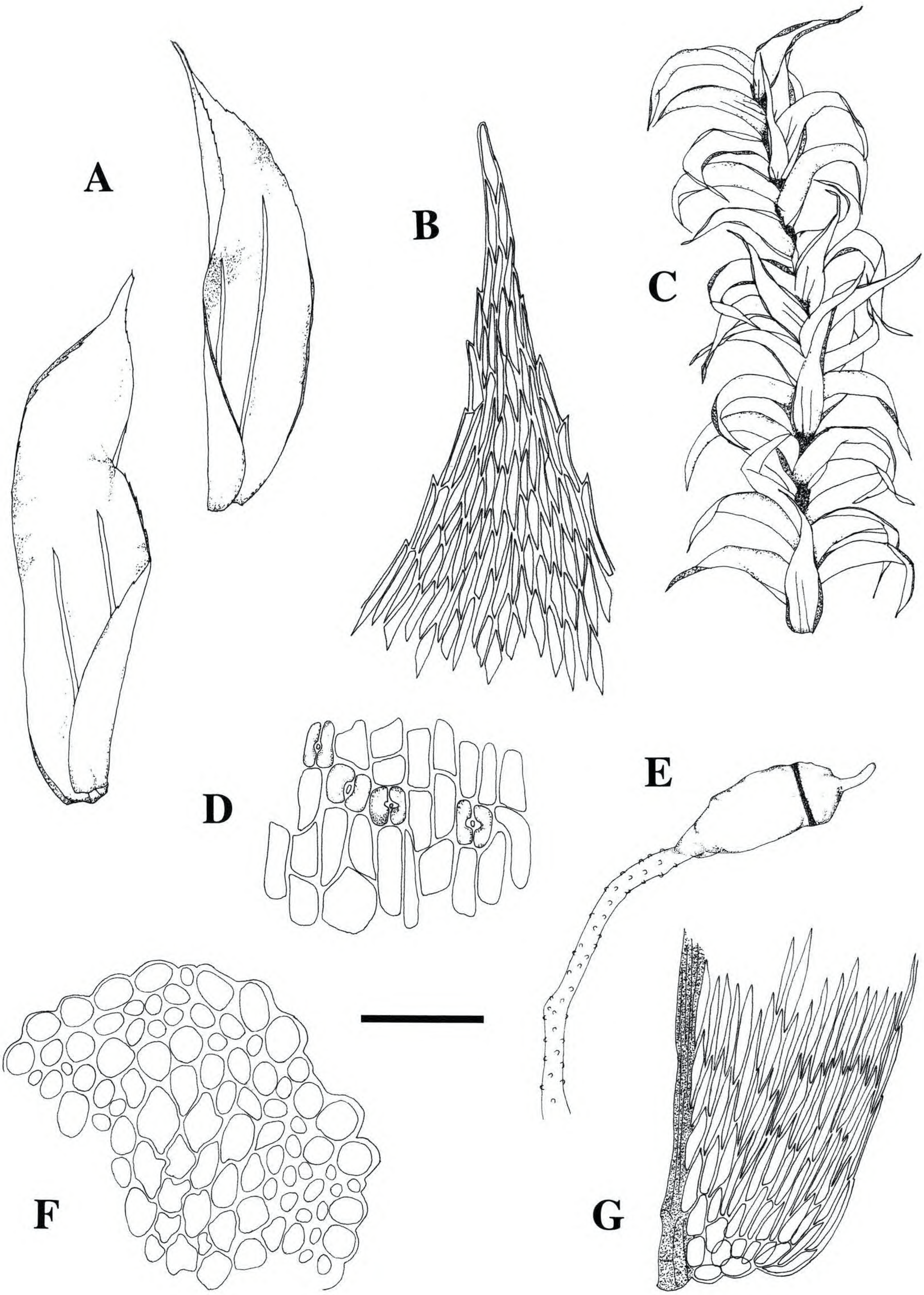


Figure 3. *Lepidopilidium devexum* (Mitt.) Broth. —A. Lateral leaves. —B. Lateral leaf apex. —C. Habit. —D. Stomata. —E. Sporophyte. —F. Transverse section of stem. —G. Basal laminal cells of lateral leaf. Scale bars: A = 0.6 mm; B, G = 0.13 mm; C = 1.96 mm; D = 0.6 mm; E = 0.8 mm; F = 0.06 mm. A, B, F, G drawn from *Brenan et al. 3910* (MO); C–E drawn from lectotype, *Mann s.n.* (NY).

and inclined capsules. Capsule orientation appears to be related to the direction the setae-bearing stems were growing at the time of collection, and particularly large specimens, such as the isotype of *L. devexum* in BM, exhibit a mixture of capsule orientations.

Additional specimens examined. CAMEROON. **Southwest:** N of Mount Koupe, above Nyassosso, ca. 15 km N of Tombel, *Magill & Crosby 9114a* (MO). GUINEA. **Nzérékoré:** Mount Nimba Strict Nature Reserve, *Allen 30751A* (MO), *Baldwin 13180* (FH), *13183* (FH). NIGERIA. **Bendel:** Okomu Forest Reserve, *Richards 3288* (MO), *3611* (MO), *3910* (MO). **Ogun:** Shasha Forest Reserve, near Akilla, *Richards 3140* (MO), *3502* (MO).

3. Lepidopilidium divaricatum (Dozy & Molk.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. Basionym: *Hookeria divaricata* Dozy & Molk., Prodr. Fl. Bryol. Surinam. 49. 13. 1854. *Lepidopilum divaricatum* (Dozy & Molk.) Mitt., J. Linn. Soc., Bot. 12: 379. 1869. TYPE CITATION: In Venezuela, juxta Rio Catuche, ubi collegit *P. W. Korthals*. TYPE: Venezuela. *Korthals s.n.* (lectotype, designated here, L!; isotypes, BM!, NY!, US!). Figure 4.

Lepidopilum corbieri Renauld & Cardot, Bull. Soc. Roy. Bot. Belgique 41(1): 85. 1905, syn. nov. *Lepidopilidium corbieri* (Renauld & Cardot) Cardot, in Grandidier, Hist. Phys. Madagascar, Mousses 413. 1915. *Hookeriopsis corbieri* (Renauld & Cardot) Broth. ex Cardot, in Grandidier, Hist. Phys. Madagascar, Mousses 413. 1915, nom. inval., pro syn. TYPE CITATION: Madagascar. Montagne d'Ambre près Diego Suarez, *Leloutre*, 1899 (Hb. Corbière). TYPE: Madagascar. Montagne d'Ambre près Diego Suarez, 1899, *Leloutre s.n.* (lectotype, designated here, PC!).

Lepidopilum subdivaricatum Renauld & Cardot, Bull. Soc. Roy. Bot. Belgique 32(1): 195. 1894. *Lepidopilidium subdivaricatum* (Renauld & Cardot) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: [Costa Rica.] s.l. (*Pitt.* No. *5658*). TYPE: Costa Rica. s. loc., *Pitt. 5658* (lectotype, designated here, PC!; isotypes, BR!, FH!, NY!).

Lepidopilidium synoicum Herzog, Biblioth. Bot. 87: 134. 1916. TYPE CITATION: [Bolivia.] An feuchten Steinen in der Quebrada de Pocona, ca. 2800 m, [*Herzog*] No. *5140*. TYPE: [Bolivia.] "Auf feuchten Steinen in der Quebrada de Pocona," ca. 2800 m, Apr. 1911, [*Herzog*] *5140* (lectotype, designated here, M!; isotypes, L!, PC!).

Plants yellowish, dull or dark green, occasionally tinted red, in lax mats; stems prostrate, to 7 cm, sparsely to irregularly branched, strongly complanate-foliate. Leaves evenly spaced, strongly dimorphic, flattened or flexuous-contorted; dorsal and ventral leaves erect, symmetric, ovate or oblong-ovate, short-acute to slenderly acuminate; lateral leaves loosely spreading, asymmetric, broadly ovate, oblong-ovate, obovate, oblong, or oblong-lanceolate, 2.2–2.7 × 1–1.3 mm, acute to abruptly and slenderly short-acuminate or occasionally long-acuminate; margins serrulate to serrate above, entire below, plane or recurved, often broadly infolded on one side at base, elimbate or bordered by 1 or 2 rows of elongate narrow cells; costae extending to midleaf or below, diverging throughout, uneven; laminal cells smooth, thin-walled; apical and median cells hexagonal to broadly rhomboidal 60–100 × 10–18 μm, becoming elongate-rhomboidal to rectangular below, basal cells enlarged, rectangular. Gemmae absent. Plants autoicous or synoicous. Setae straight or curved, 5–7.5 mm, red-brown, papillose throughout; capsules erect, suberect, or inclined, obloid-cylindrical, 0.5–1 mm long; opercula short conic-rostrate, 0.8 mm long; Spores 12–20 μm, yellow, finely papillose. Calyptrae 1.7–2 mm long.

Iconography. Dozy and Molkenboer (1854: 52, tab. 13, figs. 1–21, as *Hookeria divaricata* Dozy & Molk.); Renauld (1909: tab. 21, fig. 1, as *Lepidopilum corbieri* Renauld & Cardot); Renauld and Cardot (1915: tab. 97A, fig. 1a–e, as *Lepidopilum corbieri* Renauld & Cardot); Welch (1971: 115, figs. 173–181, as *Lepidopilidium divaricatum* (Dozy & Molk.) Broth.; 1974: 372, figs. 279–289, as *Lepidopilidium subdivaricatum* (Renauld & Cardot) Broth.); De Sloover (1975: 121, figs. 75–82, as *Lepidopilidium corbieri* (Renauld & Cardot) Cardot); Gradstein et al. (2001: 451, fig. 168A–C, as *Lepidopilidium divaricatum* (Dozy & Molk.) Broth.); Allen (2010: 545, fig. 185, as *Lepidopilidium divaricatum* (Dozy & Molk.) Broth.).

Habitat and distribution. *Lepidopilidium divaricatum* is present in southern Mexico; Central America; northern, western, and southern South America; eastern and southern Africa; and Madagascar. The species has been collected on trunks and branches of trees as well as on logs and rocks at 600–2700 m elevation (Fig. 2B).

Nomenclatural notes. Dozy and Molkenboer (1854) described *Hookeria divaricata* from an unnumbered specimen collected by Pieter Korthals in Venezuela. Duplicates of the original material are located in BM, L, NY, and US. The specimen in Dozy and Molkenboer's herbarium, deposited in L, is designated as the lectotype.

Lepidopilum corbieri was described from an unnumbered specimen collected in Madagascar by Leloutre in 1899. The type citation notes the specimen to be deposited in Corbiere's herbarium, now in CHE. Original material in Cardot's herbarium, now in PC, agrees with the specimen information cited in the 1905 protologue by Renauld and Cardot

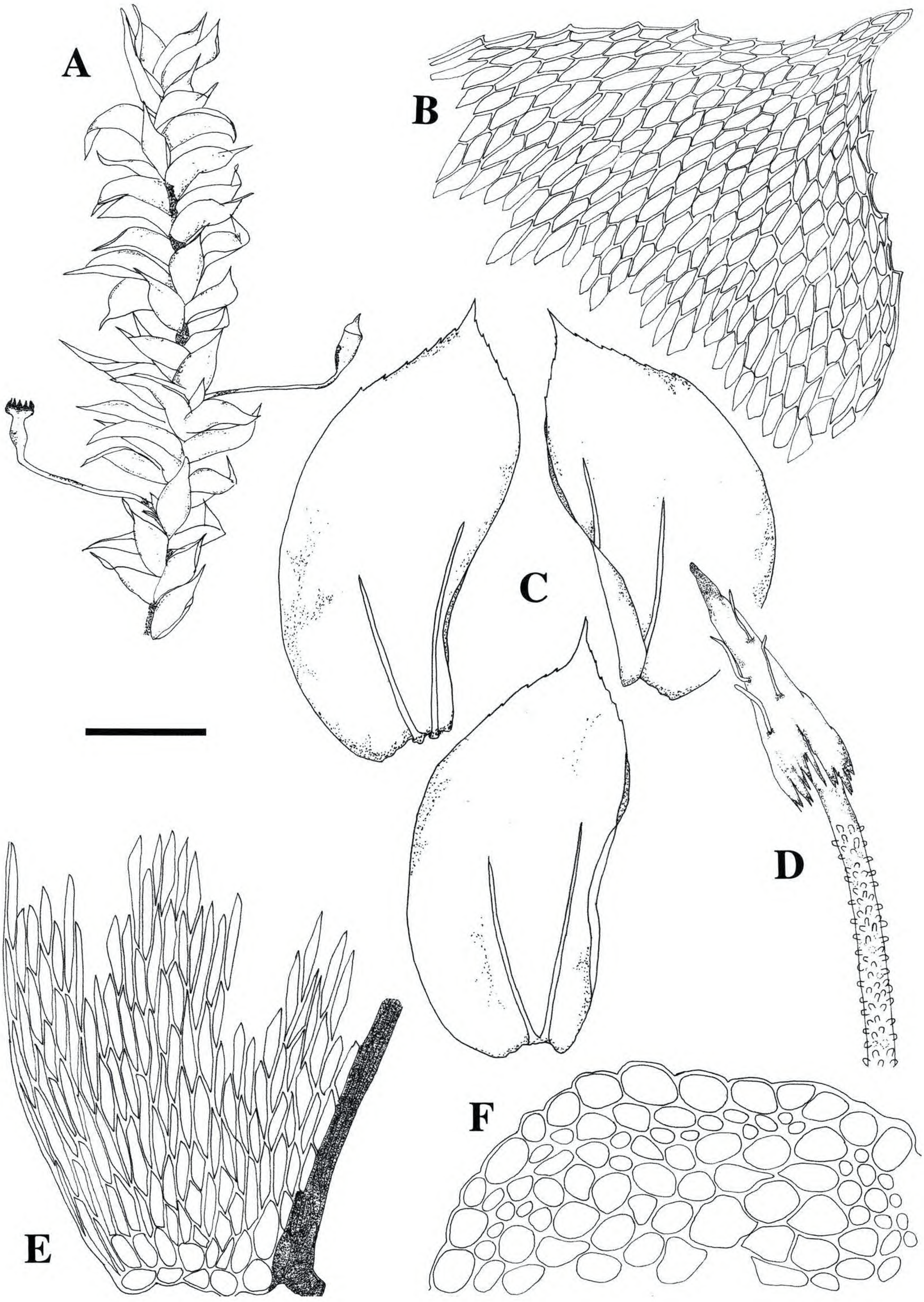


Figure 4. *Lepidopilidium divaricatum* (Dozy & Molk.) Broth. —A. Habit with sporophytes. —B. Lateral leaf apex. —C. Lateral leaves. —D. Sporophyte. —E. Basal laminal cells of lateral leaf. —F. Transverse section of stem. Scale bars: A = 1.96 mm; B, E = 0.13 mm; C = 0.6 mm; D = 0.8 mm; F = 0.06 mm. A, D drawn from *Crosby 9932* (MO); B, C, E, F drawn from *Crosby 7529* (MO).

and also has "Hb. Corbière" noted on the label. The PC specimen is designated as the lectotype.

Lepidopilum subdivaricatum was described in 1894 by Renauld and Cardot from the specimen *Pitt 5658* that was collected in Costa Rica without locality. Syntypic material is located in BR, FH, NY, and PC. The specimen in Cardot's herbarium in PC is designated as the lectotype.

Lepidopilidium synoicum was described by Herzog (1916) based on the specimen *Herzog 5140* from Bolivia. Original material of *L. synoicum* is located in L, M, and PC. Since part of Herzog's personal herbarium is deposited in M, the specimen in the author's herbarium is designated as the lectotype.

Discussion. *Lepidopilidium divaricatum* has mostly asymmetric oblong-ovate to oblong lateral leaves, although the shape can vary between broadly ovate, obovate, or oblong-lanceolate. The apices are acute to abruptly acuminate and end in short to long, slender points. The leaf margins are often obscurely bordered by one or two rows of narrow, elongate cells and are usually serrulate to serrate above. The costae extend to midleaf or below, and the apical and median leaf cells are mostly hexagonal to broadly rhomboidal. *Lepidopilidium divaricatum* is the only species in the genus currently found in both the Americas and Africa. The strongly complanate-foliate habit, autoicous or synoicous plants, and densely papillose setae separate this from all other American *Lepidopilidium* species. Among the African species, *L. divaricatum* differs from *L. devexum* in having less spreading, more contorted-flexuous leaves as well as more abruptly and slenderly acuminate leaf apices. *Lepidopilidium isleanum* has similarly oblong, short-acuminate lateral leaves with differentiated marginal borders, although it has longer (10–17 mm vs. 5–7.5 mm), flexuous setae, with a partially smooth ornamentation. *Lepidopilidium furcatum* also overlaps somewhat in lateral leaf shape and size with *L. divaricatum*. Although most specimens of *L. divaricatum* tend to have shorter and broader lateral leaf apices, specimens collected from twigs are more elongate and have apices that approach those of *L. furcatum*. Setae length and ornamentation best distinguish these two species. The setae of *L. divaricatum* are mostly longer than those of *L. furcatum* (5–7.5 mm vs. 3–5 mm) and are densely furnished with papillae. In comparison, the setae of *L. furcatum* are mostly smooth throughout, except for some scattered, low papillae present beneath the capsule.

Additional specimens examined. COSTA RICA. **Heredia:** Volcán Barba, ca. 1 km WNW of Río Ciruelas, *Crosby 9932* (MO); Las Vueltas area, *Crosby 13119* (MO), *13120* (MO). HONDURAS. **Comayagua:** San Juanillo, Cordillera de Montecillos Biol. Reserve, 12 km S of Siguatepeque, *Allen 12308* (MO), *12331* (MO); along Agua Amarilla, ca. 11 km E of Lake Yojoa, *Allen 13966* (MO). **Ocotepeque:** Montana del Candado, 13 km E of Nueva Ocotepeque & Cerro El Cocal, 12 km NE of Nueva Ocotepeque, *Allen 14488* (MO). MEXICO. **Chiapas:** Yerba Buena, Pueblo Nuevo, *Smith 2845a* (MO). PANAMA. **Chiriquí:** Valle de la Siërpe, *Salazar 5749a* (MO).

ARGENTINA. **Tucumán:** Dpto. Monteros, Río Pueblo Viejo por Quebrada del Portugues, *Churchill & Schiavone 20082* (MO), *20100* (MO). BOLIVIA. **Chuquisaca:** Belisario Boeto, Nuevo Mundo, *Lozano 957* (MO); Hernando Siles, Cañón Lacayotal, *Lozano et al. 1799* (MO); Luis Calvo, Serranía del Iñao, *Lozano & Mogro 159* (MO). **La Paz:** Santa Barbara, Apolo region, *Williams 2879* (NY). **Santa Cruz:** Florida, Mpio. Mairana, ca. 4 km NE de Las Yungas, finca de Don Lelide Cardona, *Churchill et al. 23799* (MO), *23808* (MO), *23814* (MO). **Tarija:** Arce, Reserva Natural Alarachi, Zona Alarachi, Baden Chico, *Churchill et al. 23346* (MO); Arce, Mpio. Padcaya, Cantón Emborozú, Reserva Natural Alarachi, *Churchill et al. 23459* (MO), *23463* (MO), *23474* (MO); Arce, Reserva Nac. de Flora de Fauna Tariquía Piedemonte serranía San Telmo, *Serrano et al. 5852* (MO). VENEZUELA. **Distrito Federal:** Caracas, *Ernst 12213* (MO).

DEMOCRATIC REPUBLIC OF THE CONGO. **North Kivu:** *Boutakoff s.n.* (BR). REPUBLIC OF BURUNDI. **Bururi:** Bord do lac Tanganyika, *De Sloover 19230* (BR). TANZANIA. **Dodoma:** Kiboriani Mtns., NE face of highest peak about Mpwapwa, *Pócs & Mezösi 6569/O* (MO). **Morogoro:** Uluguru Mtns., NW slopes of Palata, 9 km SSE of Morogoro, *Crosby & Pócs 8690* (MO); Uluguru Mtns., NW slopes of main ridge of Lupanga, *Pócs & Harris 6129/B* (L, MO); N Uluguru Mtns., Lupanga-Kinazi ridge, *Pócs & Mwanjabe 6836/AV* (MO). SOUTH AFRICA. **Eastern Cape:** Transkei, Mlanlane Forest Station, *van Rooy 2223* (MO). **Kwazulu-Natal:** Nkandla Forest Reserve, *Magill 5176* (MO), *5220* (MO), *5225* (MO, NY), *van Rooy 118* (MO, NY); Qudeni Forest, *Magill 5109* (MO, NY), *5145* (MO); Dhlinza Forest Reserve, *Crosby 7786* (L, MO). SWAZILAND. **Hhohho:** 5.5 km SW of Mbabane, *Kemp 1499* (MO).

4. **Lepidopilidium furcatum** (Thwaites & Mitt.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. Basionym: *Lepidopilum furcatum* Thwaites & Mitt., in Mitten, J. Linn. Soc., Bot. 13: 310. 1873. TYPE CITATION: Ins. Ceylon. *Dr. Thwaites*. TYPE: [Sri Lanka.] "Cent. Prov. Ceylon." *Thwaites s.n.* (lectotype, designated here, NY!; isotypes, BM!, FH!, G!, H!, MO!, NY!). Figure 5.

Lepidopilum hanningtonii Mitt., J. Linn. Soc., Bot. 22: 309. 1886, syn. nov. *Lepidopilidium hanningtonii* (Mitt.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: Usagara Mtns., *Bishop Hannington*. TYPE: [Tanzania.] Usagara Mtns., Oct. 1883, *Hannington s.n.* (lectotype, designated here, NY!; isotypes, BM!, NY!).

Lepidopilidium attenuatum Thér., Recueil Publ. Soc. Havraise Études Diverses 1929: 117. tab. s.n. 1930, syn. nov. TYPE CITATION: Andasibe, sur petites branches d'arbres. TYPE: Madagascar. "Forêt d'Andasibe sur l'Onive," Feb. 1925, *P. de la Bâthie s.n.* (lectotype, designated here, PC!; isotype, BR!).

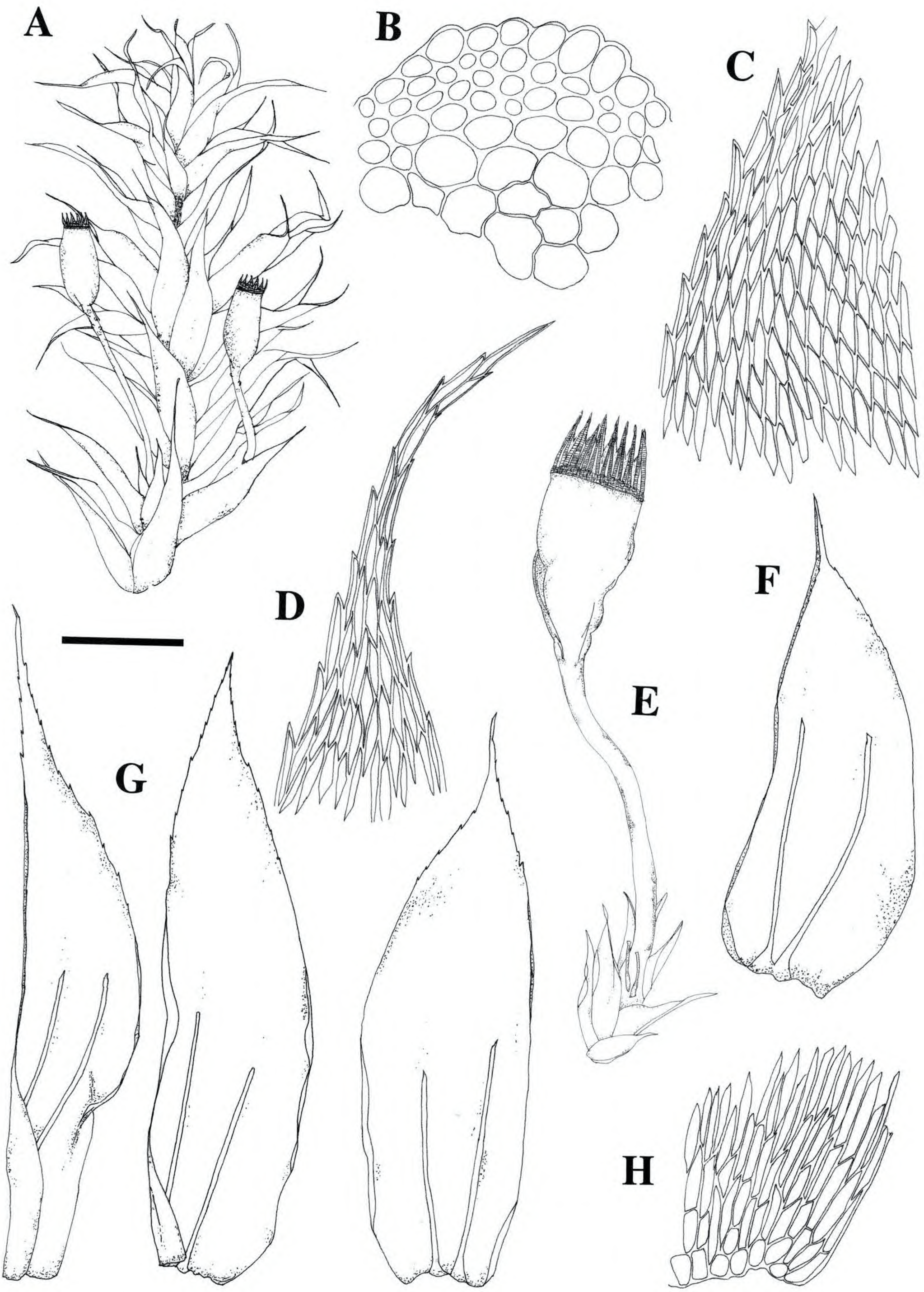


Figure 5. *Lepidopilidium furcatum* (Thwaites & Mitt.) Broth. —A. Habit with sporophytes. —B. Transverse section of stem. —C. Median laminal cells of lateral leaf. —D. Lateral leaf apex. —E. Sporophyte. —F. Dorsal leaf. —G. Lateral leaves. —H. Basal laminal cells of lateral leaf. Scale bars: A = 1.96 mm; B = 0.06 mm; C, D, H = 0.13 mm; E = 0.8 mm; F, G = 0.6 mm. A, F, G drawn from *Crosby 14437* (MO); B–D, H drawn from *De Sloover 12913* (MO); E drawn from lectotype, *Bequaert 4743 bis* (BR).

Lepidopilidium theriotii Naveau, in Dixon & Thériot, Mélanges Bryol. Lichénol. 74. 1942, syn. nov. TYPE CITATION: Ruvenzori, Lanuri, 2.500 m, sur bamboo; leg. Dr. *Bequaert*, mai 1914 [*Bequaert*]. TYPE: [Democratic Republic of the Congo.] Ruvenzori (Lanuri) ± 2500 m, sur bambou, 18 May 1914, *Bequaert 4743 bis* (lectotype, designated here, BR!; isotypes, BR!, JE!, PC!).

Lepidopilidium subdevexum (Broth.) Broth. var. *intermedium* P. de la Varde, Rev. Bryol. Lichénol. 11: 183. 1939, syn. nov. TYPE CITATION: [l'Oubangui, Région de Berbérati] Sur brindilles près d'un ruisseau, forêt de la mission [*R. P. C. Tisserant*]. TYPE: [Central African Republic.] Oubangui, sur rameaux et feuilles au dessus de l'eau ruisseau dans la forêt de Berbérati, 6 June 1937, *R. P. Tisserant s.n.* (lectotype, designated here, PC!; isotypes, BR!, PC!).

Plants glossy, yellowish green to golden, sometimes tinted red, in loose to dense mats or tufts; stems prostrate or erect, to 7 cm, frequently branched, moderately complanate-foliate. Leaves crowded, strongly dimorphic; dorsal and ventral leaves erect to erect-spreading, symmetric, ovate-oblong to oblong-lanceolate, abruptly acuminate; lateral leaves spreading to wide-spreading, asymmetric, ovate-lanceolate, oblong, oblong-lanceolate, or lanceolate, 2.6–4 × 0.6–1 mm, narrowly short- to long-acuminate, sometimes tapering into a long point; margins serrate to sharply serrate above, becoming serrulate to entire below, plane or narrowly recurved, elimbate; costae extending to midleaf or above, sometimes projecting at the tips; laminal cells smooth, thin-walled; apical and median cells rhomboidal to narrowly or broadly fusiform, 60–100 × 10–18 μm, sometimes linear-elongate becoming oblong-rectangular to oblong-fusiform below, basal cells enlarged, rectangular. Gemmae present. Autoicous or synoicous. Setae straight or curved, 3–5 mm, red, smooth throughout or smooth below to lightly or densely papillose above. Capsules erect, sub-erect, or inclined, obloid, 1–1.3 mm long; opercula conic, 0.6 mm long. Spores 17–25 μm, yellow, finely papillose. Calyptrae 1.7–2 mm long.

Iconography. Abeywickrama (1960: 96, figs. 112a–c, as *Lepidopilidium furcatum* (Thwaites & Mitt.) Broth.); Thériot (1930: 118, figs. 1–10, as *L. attenuatum* Thér.); De Sloover (1975: 119, figs. 65–74, as *L. theriotii* Naveau); Magill and van Rooy (1998: 610, fig. 169, 1–13, as *L. hanningtonii* Mitt.).

Habitat and distribution. *Lepidopilidium furcatum* is known across central and south tropical Africa; southern Africa; Madagascar; southern India, and Sri Lanka. It has been collected on the trunks, branches, and twigs of trees and shrubs, also on leaves and vines, and occasionally on moist boulders at 300–2300 m elevation (Fig. 6A).

Nomenclatural notes. Thwaites and Mitten described *Lepidopilum furcatum* in 1873 from an unnumbered collection made by Thwaites in Sri Lanka. Original material is deposited in BM, FH, G, H, MO, and NY, among duplicates. A duplicate in Mitten's herbarium in NY includes accompanying illustrations and is designated as the lectotype.

Lepidopilum hanningtonii was described by Mitten based on an unnumbered collection made by Hannington in Tanzania. A particularly large specimen in NY, with accompanying illustrations, is designated as the lectotype.

Thériot (1930) described *Lepidopilidium attenuatum* based on a collection made by Perrier de la Bâthie in Madagascar. Syntypic material is deposited in BR and PC. The specimen in PC is large and has intact sporophytes. It is designated as the lectotype.

Original material of *Lepidopilidium theriorii* is deposited in BR, JE, and PC. A particularly large specimen in BR is designated as the lectotype.

Lepidopilidium subdevexum var. *intermedium* was described from a collection made by Tisserant in what is present-day Central African Republic. Duplicates of original material are deposited in BR and PC. The larger of the duplicates in PC is designated as the lectotype.

Discussion. Most specimens of *Lepidopilidium furcatum* form loosely complanate mats with frequently branched stems, although some specimens form dense tufts of erect branches. Elizabeth Britton's notes on the specimen packet of the lectotype of *L. furcatum* (NY) call attention to the mixture of these two unlike growth forms. The lateral leaves in both are similarly oblong-lanceolate and have narrowly long-acuminate apices. The sporophytes of *L. furcatum* have short setae and obloid capsules. Both the length and the ornamentation of the setae vary between specimens. The types of *L. hanningtonii* and *L. theriotii* have nearly smooth setae throughout, whereas the type of *L. attenuatum* has setae that are smooth below and lightly papillose above.

As noted by De Sloover (1975), sterile specimens of *Lepidopilidium furcatum* are exceedingly difficult to separate from *Lepidopilum lastii* Mitt. Both species are similar in their complanate-foliate habit, size, and leaf shape, although *L. lastii* tends to be slightly larger and have longer, less densely branched stems. Sterile specimens of *Lepidopilidium furcatum* can also be confused with *Thamniopsis pappeana* (Hampe) W. R. Buck. Ochyra and Pócs's (1992) *Bryophyta Africana Selecta* specimens No. *93*, as *Lepidopilidium han-*

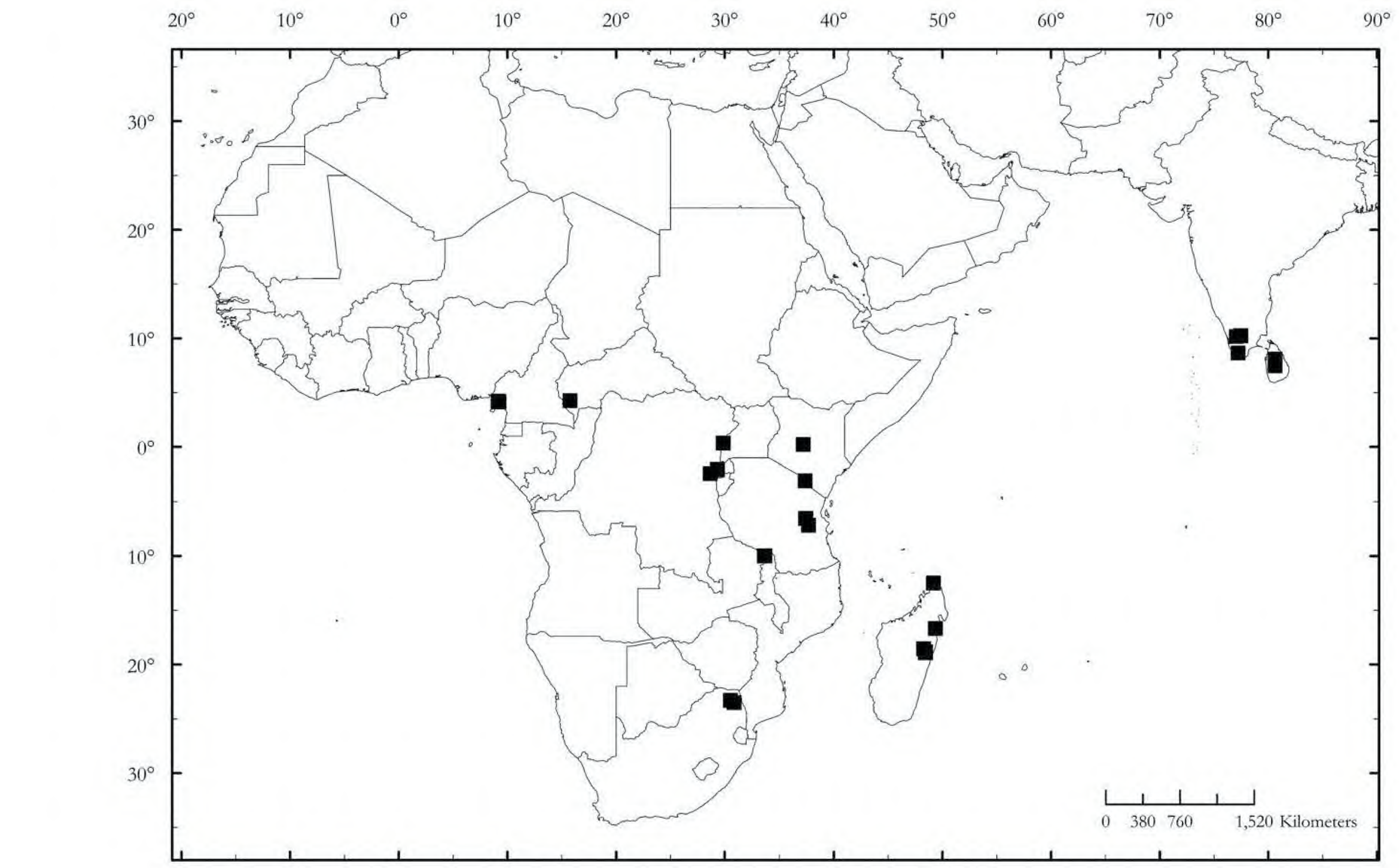


Figure 6. Distributions of *Lepidopilidium* species. —A. *Lepidopilidium furcatum* (Thwaites & Mitt.) Broth. (■). —B. *Lepidopilidium nitens* (Hornsch.) Broth. (●).

ningtonii, and residual specimens distributed from Foreau's *Musci Madurenses Indiae Meridionalis Exsiccati*, as *Lepidopilidium furcatum*, belong to *T. pappeana*. *Lepidopilidium furcatum* and *T. pappeana* are superficially similar, but differ in that *T. pappeana* has inflated, bifurcate teeth on the leaf margins.

Additional specimens examined. CAMEROON. **Southwest:** supra Buea, *Bornmüller 8* (NY); 1891, *Junger s.n.* (H). DEMOCRATIC REPUBLIC OF THE CONGO. **Sud Kivu:** *De Sloover 12913* (BR, MO), *12920* (BM, MO), *12941B* (BR, MO). KENYA. **Central:** Mt. Kenya Natl. Forest, S slopes of Mt. Kenya, above Castle Forest Station, *Crosby & Crosby 8732* (MO). MADAGASCAR. **Antsiranana:** Parc Natl. Montagne d'Ambre, *Touw & Snoek 25108* (L). **Toamasina:** Tamatave, 3 km SE of Perinet, *Crosby 14434* (MO); valley of Marimbona River, W of Soanierano, *Lam & Meeuse 5929A* (L); Périnet Réserve Forestiere at Andsibe, *Magill et al. 9616* (MO), *9636* (MO). MALAWI. July 1896, *Whyte s.n.* (H). RWANDA. **Cyangugu:** Uwinka, forêt de Rugege, rivière Buruli, *De Sloover 18839* (MO, NY, S). SOUTH AFRICA. **Transvaal:** *Crosby & Crosby 7612* (MO); *7617* (MO); *7662* (MO). TANZANIA. **Morogoro:** *Crosby et al. 8668* (BR, L, MO), *8669* (MO), *8679* (MO, L), *8689* (MO), *8693* (MO), *8700* (MO), *8704* (MO), *14437* (MO); *Pócs et al. 6220/D* (MO). **Ulanga:** Sanje, *Thomas 3762A* (NY).

INDIA. **Tamil Nadu:** Madras State, Madura Distr., Palni Hills, Kodaikanal, 1929, *Foreau s.n.* (US); *Daniels & Kariyappa 3627 p.p.* (MO). SRI LANKA. **Central:** Feb. 2006, *Herzog s.n.* (H), *Herzog 47/a* (JE); *Naylor Beckett C169* (H). **North Central:** Ritigala, *Naylor Beckett 89* (NY).

5. **Lepidopilidium isleanum** (Besch.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3, II): 944. 1907. Basionym: *Lepidopilum isleanum* Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 279. 1880. TYPE CITATION: La Réunion: entre les branches de Calamagrostis (?) Belous, 25 mai 1874, *G. de l'Isle* no. *183*. TYPE: La Réunion. *G. de l'Isle 183* (lectotype, designated here, BM!; isotypes, NY!, S!). Figure 7.

Lepidopilum flexuosum Besch., Ann. Sci. Nat., Bot., sér. 6, 10: 280. 1880, syn. nov. *Lepidopilidium flexuosum* (Besch.) Broth. ex Paris, Coll. Nom. Broth. 17. 1909. TYPE CITATION: La Réunion: plaine des Palmistes, *G. de l'Isle*, no. *293*. Rare. TYPE: La Réunion. Plaine des Palmistes, *G. de l'Isle 293* (lectotype, designated here, BM!; isotype, NY!).

Lepidopilidium parvulum Schimp. ex Cardot, in C. H. Wright ex Cardot, in Grandidier, Hist. Phys. Madagascar, Mousses 414. 1915, syn. nov. *Lepidopilum parvulum* Schimp., in C. H. Wright, J. Bot. 26: 266. 1888, nom. inval. [lacks description]. TYPE CITATION: Madagascar. (Nord-Ouest), sans indication de localité (*Pervillé*: herb. Jard. Bot. Kew). TYPE: Madagascar. s. loc. *Pervillé M. par. 52* (lectotype, designated here, BM!; isolectotype, NY!).

Lepidopilum subrevolutum Renauld & Cardot, in Renauld, Suppl. Prodr. Fl. Bryol. Madagascar 20: 80, tab. 20, fig. 2. 1909, syn. nov. *Lepidopilidium subrevolutum* (Renauld & Cardot) Cardot, in Grandidier, Hist. Phys. Madagascar, Mousses 414. 1915, tab. 97A, fig. 3a–e. TYPE CITATION: Madagascar. forêt de Analamazaotra, Rev. *Camboué*, 1890. TYPE: Madagascar. *P. Camboué s.n.* (lectotype, designated here, PC!; isotype, PC!).

Plants glossy, pale green to golden-yellow, sometimes tinted red, in dense mats; stems prostrate, to 3 cm, sparsely branched, complanate-foliate. Leaves not or slightly dimorphic, contorted when dry; dorsal and ventral leaves symmetric, mostly ovate to oblong-ovate, 1.6–2.5 × 0.7–1 mm, abruptly short-acuminate; lateral leaves slightly asymmetric, oblong-ovate or oblong, 1.8–3 × 0.6–1.1 mm, abruptly acuminate with short, slender tips, rounded to the insertion; margins serrulate to serrate above, becoming serrulate to entire below, plane or narrowly recurved, obscurely bordered by 1 or 2 rows of narrow, elongate cells, confluent at apices; costae extending to midleaf, diverging throughout, uneven, not projecting at apices; laminal cells smooth, thick-walled, sometimes porose, apical and median cells oval-hexagonal to narrowly or broadly rhomboidal, 40–72 × 18–22 μm, becoming elongate-rhomboidal below, basal cells enlarged, rectangular. Gemmae present. Plants autoicous. Setae flexuous, 7–15 mm, yellow to red, lightly papillose below to smooth, lightly or densely papillose above, sometimes smooth; capsules suberect to inclined, sometimes horizontal, obloid to obloid-cylindric, 1.3–2 mm long, sometimes curved, often constricted below the mouth; opercula conic-rostrate, 1 mm long. Spores 25–32 μm, yellow, finely papillose. Calyptrae 2–2.5 mm long.

Iconography. Renauld (1909: tab. 20, fig. 2a–e, as *Lepidopilum subrevolutum* Renauld & Cardot): Renauld & Cardot (1915: tab. 97A, fig. 3a–e, as *Lepidopilum subrevolutum* Renauld & Cardot).

Habitat and distribution. *Lepidopilidium isleanum* is known from the type collections and a few additional specimens from Madagascar and La Réunion, where it has been collected on wet rocks, logs, and tree trunks and branches at 1500–1965 m elevation (Fig. 2A).

Nomenclatural notes. *Lepidopilum isleanum* and *L. flexuosum* were described simultaneously by Bescherelle (1880) from two of de l'Isle's specimens (*183* and *293*, respectively) that were collected on the island of La Réunion. Syntypic material of each species is deposited in Bescherelle's herbarium, now in BM. These are designated as lectotypes.

The type of *Lepidopilidium parvulum* was described from specimens collected by Pervillé in Madagascar. Two specimens matching the type citation were found in BM, neither of which are annotated by Cardot. Although no collection number was cited in the protologue, both BM specimens have numbers (*Pervillé*

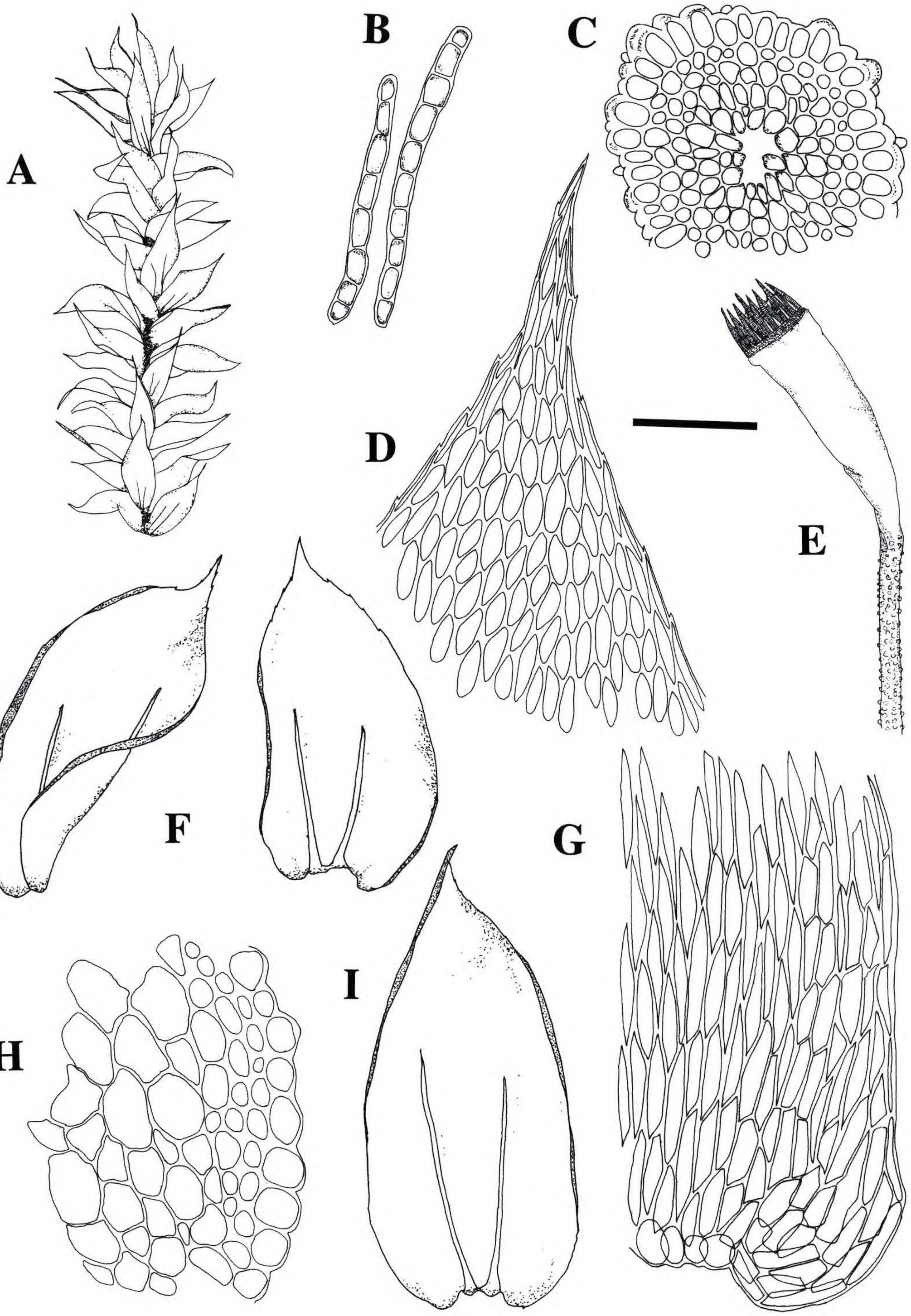


Figure 7. *Lepidopilidium isleanum* (Besch.) Broth. —A. Habit. —B. Gemmae. —C. Transverse section of seta. —D. Lateral leaf apex. —E. Sporophyte. —F. Lateral leaves. —G. Basal laminal cells of lateral leaf. —H. Transverse section of stem. —I. Dorsal leaf. Scale bars: A = 1.96 mm; B, D, G = 0.13 mm; C, H = 0.06 mm; E = 0.8 mm; F, I = 0.6 mm. A drawn from *Carter 8057* (NY); B, D, F–I drawn from *Arts RÉU59/29* (BR); C, E drawn from syntype *Perville 41* (BM).

41 and *Pervillé M. par. 52*, respectively). Except for their setae ornamentation, the specimens appear to be morphologically identical. *Pervillé 41* has setae that are smooth below and densely papillose above, whereas specimen *Pervillé M. par. 52* has setae that are smooth below and mostly smooth above, with a few low, scattered papillae. The original description of *L. parvulum* notes the setae ornamentation to be smooth throughout. Since *Pervillé M. par. 52* more closely matches the setae ornamentation noted in the type description, it is considered to be the more appropriate specimen as lectotype.

Renauld and Cardot (1909) described *Lepidopilum subrevolutum* based on an unnumbered Malagasy collection made by Camboué in 1890. Three duplicates of original, syntypic material are deposited in PC. The largest of these is designated the lectotpye.

Discussion. *Lepidopilidium isleanum* has slightly asymmetrical, oblong-ovate to oblong lateral leaves that are rounded to the insertion. The leaves have oval-hexagonal to narrowly or broadly rhomboidal median cells, and are often bordered by one or two rows of narrowly elongate cells, best visible in the slender, abruptly short-acuminate apices. Perhaps the most noteworthy character of *L. isleanum* is its elongate (7–15 mm), flexuous setae.

Bescherelle (1880) described *Lepidopilum isleanum* alongside the name *L. flexuosum* for two specimens collected on La Réunion by de l'Isle. The setae of *L. isleanum* [≡ *Lepidopilidium isleanum*] were described as smooth below and smooth to lightly papillose above, whereas the setae of *L. flexuosum* [≡ *Lepidopilidium flexuosum*] were described as smooth to papillose below and densely papillose above.

Similarly, Renauld and Cardot (1915) treated two morphologically similar *Lepidopilidium* names from Madagascar, *L. subrevolutum* and *L. parvulum*, which differ only in their setae ornamentation as well. Renauld and Cardot's (1915) illustrations of *L. subrevolutum* (as *Lepidopilum subrevolutum*) depict the densely papillose setae. On the other hand, *L. parvulum*, which was originally described as having smooth setae, has some setae with distally scattered papillae. An examination of additional specimens from Madagascar and La Réunion found setae with variably scattered papillae as well, i.e., *Carter et al. 8057* (NY) and *Arts RÉU59/29* (BR). Although only a limited number of specimens, including types, were available for study, the setae ornamentation differences noted in the original descriptions are considered to be extremes in a continuum.

Additional specimens examined. LA RÉUNION. Cilaos, Cirque de Cilaos, *Carter et al. 7952* (NY), *8055* (NY), *8057* (NY), *Arts RÉU59/29* (BR); Salazie, Cirque de Salazie, *Carter 7952* (NY), *Arts RÉU07/31* (BR). MADAGASCAR. *Pervillé 41* (syntype, *Lepidopilidium parvulum*, BM!). **Fianarantsoa:** *Onraedt 71.m.5405* (BR).

6. **Lepidopilidium nitens** (Hornsch.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. Basionym: *Hookeria nitens* Hornsch., Fl. Bras. 1(2): 65. 1840. TYPE CITATION: Ad truncos putridos silvarum umbrosarum prope Novo-Friburgum in societate *Lepidopili subnervis*; Decembri, c. fr. maturis, *Beyrich*. TYPE: Brasilia prope Novo Friburgum, *Beyrich s.n.* (lectotype, designated here, BM [barcode BM000862091]!; isotype, BM!). Figure 8.

Plagiothecium regnellii Ångstr., Öfvers. Förh. Kongl. Svenska Vetensk.-Akad. 33(4): 44. 1876, syn. nov. *Lepidopilidium regnellii* (Ångstr.) W. R. Buck & Ireland, Fl. Neotrop. Monogr. 50: 19. 1989. TYPE CITATION: Brazil. Caldas, *Regnell 15* transmisit. TYPE: Brazil. Caldas, *A. F. Regnell 15* (lectotype, designated by Buck & Ireland, 1989: 19, S!; isolectotype, S!).

Lepidopilum laevisetum Hampe, Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn ser. 4, 1: 124. 1879, syn. nov. *Lepidopilidium laevisetum* (Hampe) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: [Brazil.] Prope Rio de Janeiro: *Glaziou* (*9235*, *parce*) TYPE: [Brazil.] Rio de Janeiro, *Glaziou 9235* (lectotype, designated here, BM!; isotypes, BM!, BR!, G!, PC not seen).

Hookeria aureopurpurea Geh. & Hampe, Flora 64: 409. 1881, as "aureo-purpurea," syn. nov. *Lepidopilidium aureopurpureum* (Geh. & Hampe) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: [Brazil.] Prope Apiahy, Junio 1879, *Puiggari* (*179*, *192*). TYPE: Brazil. "Brasilia: prope Apiahy, S. Paulo," 1879, *G. L. T. Puiggari s.n.* (lectotype, designated here, BM [barcode BM000862101]!).

Hookeria subnitens Geh. & Hampe, Flora 64: 409. 1881. *Lepidopilidium subnitens* (Geh. & Hampe) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: [Brazil.] Prope Apiahy, Aprili–Mayo 1879, *Puiggari*; prope Iporanga, statu sterili, foliis serrate-dentatis: idem. TYPE: [Brazil.] "Brasilia prope Apiahy, S. Paulo," 1879, *G. L. T. Puiggari* s.n. (lectotype, designated here, BM [barcode BM000862089]!).

Hookeria subnitens var. *latior* Geh. & Hampe, Flora 64: 409. 1881, syn. nov. TYPE CITATION: [Brazil.] Prope Apiahy, Majo 1879, *Puiggari* (*538*). TYPE: Brazil, São Paulo, May 1879, *Puiggari 538* (lectotype, designated here, BM!).

Lepidopilum mariei Besch. ex Kindb., Enum. Bryin. Exot., suppl. 2, 100. 1891, nom. inval. [lacks description]. TYPE: Guadeloupe, *Marie 145* (type, NY!).

Hookeria entodontella Müll. Hal. ex Broth., Bih. Kongl. Svenska Vetensk.-Akad. Handl. 21. 3(3): 40. 1895, syn. nov. *Hookeria entodontella* Müll. Hal., Hedwigia 39: 279. 1900, nom. illeg. *Lepidopilidium entodontella* (Müll. Hal. ex Broth.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: [Brazil.] Prov. S. Paulo, Santos, Sororocaba, in

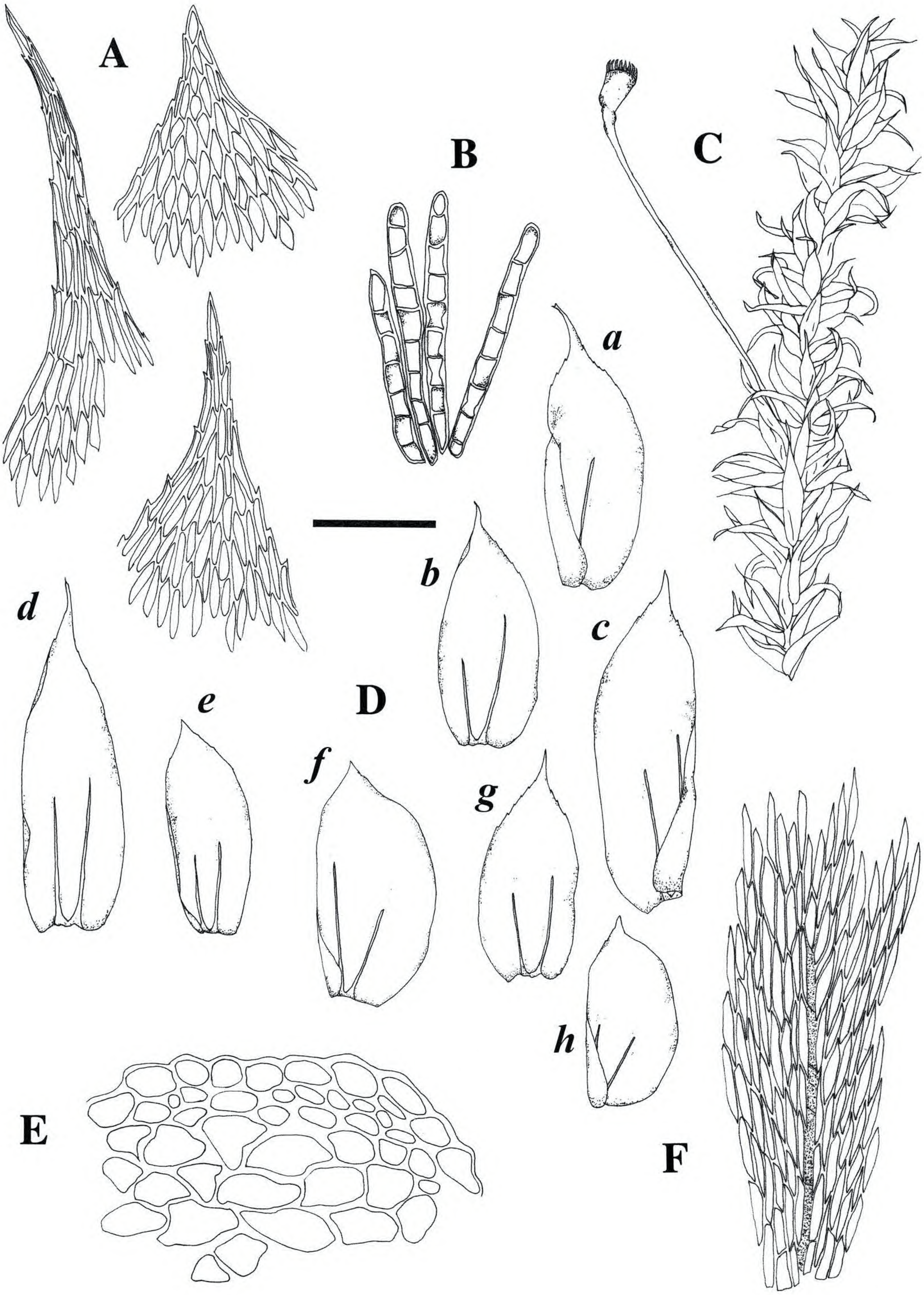


Figure 8. *Lepidopilidium nitens* (Hornsch.) Broth. —A. Lateral leaf apices. —B. Gemmae. —C. Habit with sporophyte. —D. Lateral leaves (*a–h*). —E. Transverse section of stem. —F. Median laminal cells of lateral leaf. Scale bars: A, B, F = 0.13 mm; C = 1.96 mm; D = 0.6 mm; E = 0.06 mm. A drawn from *Steyermark 103395* (MO); B, C, D*b*, E, F, drawn from lectotype, *Ule 1470* (S); D*a* drawn from *Richards 309* (U); D*c* drawn from *Elliott 206a* (BM); D*d* drawn from *Hoehne 122* (JE); D*e* drawn from *Glaziou 7090* (BM); D*f* drawn from *Britton 6581* (NY); D*g* drawn from *Allen 15115* (MO); D*h* drawn from lectotype, *Regnell 15* (S).

adscensu montis Morro de Curupira, 500 m (*Mosén n. 72*). TYPE: [Brazil.] "S[ão] Paulo, Santos, Sororocaba, in adscensu montis Morro de Curupira," 500 m, 30 Mar. 1875, *Mosén 72* (lectotype, designated here, S [barcode S-B121809]!; isotypes, H!, S!).

Crossomitrium portoricense Müll. Hal., Hedwigia 37: 244. 1898, as "Portoricense," syn. nov. *Lepidopilum portoricense* (Müll. Hal.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 956. 1907. *Lepidopilidium portoricense* (Müll. Hal.) H. A. Crum & Steere, Bryologist 59: 253. 1956. TYPE CITATION: Portorico, prope Aybonito, ad arbores sylvae primaevae montis Cuyon, Nov. 1885, *P. Sintenis* in Hb. Krug et Urban. TYPE: [Puerto Rico.] "prope Aybonito, *P. Sintenis*" *Plantae Portoricenses*. F62, Aybonito, 3 Nov. 1885 (lectotype, designated here, BM!, isotypes, G!, NY!, S!).

Hookeria longicuspis Müll. Hal., Hedwigia 39: 273. 1900. *Lepidopilum longicuspis* Müll. Hal., in Ule, Hedwigia 38 (Beibl.): 59. 1899, nom. inval. [lacks description]. *Lepidopilidium longicuspis* (Müll. Hal.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: Brasilia, Sa. Catharina, Nova Venezia, ad truncos arborum sylvestrium, Julio 1891, *E. Ule*, Coll. No. *1030*. TYPE: [Brazil.] Santa Catarina, "Nova Venezia, ad truncos silvae," July 1891, *E. Ule s.n.* (lectotype, designated here, G!; isotypes, BM!, BR!, G!, JE!, L!, M!).

Hookeria tenuiseta Müll. Hal., Hedwigia 39: 272. 1900. *Lepidopilidium tenuisetum* (Müll. Hal.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: Brasilia, Sa. Catharina, colonia Blumenau, ad trunculum arboris sylvestris prope rivulum Mulde, Julio 1888, *E. Ule*, Coll. No. *317*; Joinville, in sylva primaeva, Junio 1888, sterilis: idem, No. *468*. TYPE: [Brazil.] "Brasilia, Blumenau, an einem Stämmchen im Walde an der Mulde," July 1888, *E. Ule 317* (lectotype, designated here, H!).

Hookeria gracilifrons Müll. Hal., Hedwigia 39: 279. 1900. syn. nov. *Lepidopilidium gracilifrons* (Müll. Hal.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: Brasilia, Minas Geraës, Ouro Preto, ad truncos arborum, Martio 1892, *E. Ule*, Coll. No. *1470*. TYPE: Brazil. "Brasilia, Minas Gerais: Ouro Preto," 1892, *E. Ule 1470* (lectotype, designated here, S!; isotype, US!).

Hookeria wainioi Broth., Acta Soc. Sci. Fenn. 19(5): 19. 1901, syn. nov. *Lepidopilidium wainioi* (Broth.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: Minas Gerais, Caraça; planta mascula [*E. Wainio*]. TYPE: Brazil. "Brasilia, Minas Geraës, Caraça," 1885, *E. Wainio s.n.* (lectotype, designated here, S!).

Plants glossy, green to golden-yellow, sometimes tinted red, in dense to lax mats; stems prostrate, to 7 cm, irregularly branched, moderately to strongly complanate-foliate. Leaves evenly or densely spaced, strongly dimorphic; dorsal and ventral leaves appressed or erect-spreading when dry or wet, symmetric, elliptical, ovate, oblong-ovate, ovate-lanceolate, or oblong, 1–2.5 × 0.3–0.8 mm, abruptly acute, short-acuminate, or broadly acuminate; lateral leaves flat or shallowly concave, erect-spreading to falcate-spreading when dry, erect-spreading to wide-spreading, sometimes flexuous when wet; polymorphic: asymmetric, ovate, 1–1.3 × 0.5–0.6 mm, abruptly acute; or symmetric to asymmetric, oblong-ovate to oblong, 1.1–1.9 × 0.3–0.6 mm, acute to abruptly short-acuminate; or symmetric, oblong to oblong-lanceolate, 1.7–2.1 × 0.5–0.7 mm, narrowly long-acuminate; or symmetric, oblong-lanceolate, 2–2.6 × 0.4–0.7 mm, broadly long-acuminate; margins entire, serrulate, or serrate above, serrulate to entire below, plane or narrowly recurved, sometimes broadly incurved on one side at base, not or narrowly bordered by one or two rows of long, narrow cells; costae extending below, to, or above midleaf, parallel or diverging throughout, uneven, smooth at back, not projecting or weakly projecting at tips; laminal cells smooth, thin-walled, sometimes porose; apical and median cells hexagonal, rhomboidal, elongate-rhomboidal, or narrowly to broadly fusiform, 40–100 × 10–20 μm, becoming longer and narrow below; basal cells enlarged, short to long-rectangular. Gemmae present. Plants dioicous. Setae straight, 7–15 mm long, red-orange, smooth or sometimes lightly papillose above; capsules suberect to inclined, obloid-cylindric, 1.2–1.8 mm long, neck well-developed; opercula long conic-rostrate, 1 mm long. Spores 15–25 μm, yellow, finely papillose. Calyptrae 1.6–2 mm long.

Iconography. Welch (1969: 120, figs. 164–168, as *Lepidopilidium portoricense* (Müll. Hal.) H. A. Crum & Steere); Sehnem (1979: 77, tab. IX, fig. a, 1–7, as *L. gracilifrons* (Müll. Hal.) Broth.; fig. c, 1–5, as *L. laevisetum* (Hampe) Broth.); Florschütz-de Waard (1986: 332, fig. 130, as *L. portoricense* (Müll. Hal.) H. A. Crum & Steere); Bowers (1994: 818, fig. 616, as *L. portoricense* (Müll. Hal.) H. A. Crum & Steere); Duarte Bello (1997: 623, tab. 249, as *L. portoricense* (Müll. Hal.) H. A. Crum & Steere); Buck (1998: 44, tab. 16, figs. 1–8, as *L. portoricense* (Müll. Hal.) H. A. Crum & Steere; 2003: 100, fig. 91, as *L. portoricense* (Müll. Hal.) H. A. Crum & Steere); Peralta & Yano (2006: 59, fig. 7g–k, as *L. portoricense* (Müll. Hal.) H. A. Crum & Steere); Yano and Pôrto (2006: 18, figs. 9–12, as *L. portoricense* (Müll. Hal.) H. A. Crum & Steere); Vaz and Costa (2006: 979, figs. 7–14, as *L. brevisetum* (Hampe) Broth.; 979, figs. 15–20, as *L. laevisetum* (Hampe) Broth.); Vaz-Imbassahy and Costa (2008: 562, figs. 96–103, as *L. brevisetum* (Hampe) Broth.; 562, figs. 104–109, as *L. laevisetum* (Hampe) Broth.); Allen (2010: 547, fig. 186, as *L. portoricense* (Müll. Hal.) H. A. Crum & Steere).

Habitat and distribution. *Lepidopilidium nitens* is a widespread Neotropical species present in southern Mexico, Central America, the Caribbean, northern South America, and Brazil. It is an epiphyte noted as

collected from tree roots, bases, trunks, and branches, and branchlets as well as treelets, low shrubs, and lianas. The species is also epiphyllous. It occasionally occurs on decaying logs, stumps, and rocks at 420–1600 m elevation (Fig. 6B).

Nomenclatural notes. *Hookeria nitens* is the oldest name now placed in *Lepidopilidium*. The species was described from a collection made by Beyrich in Brazil. Three specimens of original, syntypic material are in BM, mounted on a single herbarium sheet alongside a non-typic Glaziou specimen (*7090*). The herbarium sheet also includes notes by Hampe concerning *Hookeria* sect. *Lamprophyllum*. The specimen bearing the label "ex herb. Hornschuch" is selected as the lectotype.

Hampe (1879) described *Lepidopilum laevisetum* based on the specimen *Glaziou 9235* from Rio de Janeiro, Brazil. Original material now resides in BM, BR, G, and PC, and each specimen has an original label with "Rio Janeiro" and "Glaziou" stamped on the bottom. Across the top of these labels is the name "Lepidopilum laevisetum," somewhat illegibly written in Hampe's handwriting. On specimens in BM and BR, the epithet "laevisetum" has been misread and reprinted on the herbarium sheet as "brevisetum," although the enclosed specimens do not match the type of *Lepidopilidium brevisetum*. The specimen *Glaziou 2935* in PC has a numbering error on the label that is incorrectly rewritten for *Glaziou 9235*. The specimen in Hampe's herbarium in BM is large and therefore designated as the lectotype.

The type citation for *Hookeria aureopurpurea* includes Puiggari's collection numbers *179* and *192*, although neither of these numbers were found during this study despite two attempts at locating them. However, an unnumbered specimen of *H. aureopurpurea*, annotated as "sp. nov." and marked as from Geheeb's herbarium as well as from Hampe's herbarium, now in BM, is original material. That specimen is designated as the lectotype.

Hookeria subnitens was described by Geheeb and Hampe (1881) from specimens collected by Puiggari in 1879 near Apiahy and Iporanga, Brazil. An unnumbered Puiggari specimen in BM noted from Geheeb's herbarium (as well as from Hampe's herbarium) is annotated as "sp. nov." and matches the type citation for the Apiahy locality except for the months of collection specified in the protologue as "Aprili–Majo 1879." Puiggari specimens in JE, L, and S have these months of collection on their labels but have the collection number *540* associated with them. Similarly, specimens in BM, H, and JE match the protologue information for the Iporanga locality but have the collection number *929* associated with them. No collection numbers are cited in the protologue. The specimen from Geheeb's herbarium in BM is selected as the lectotype.

Geheeb and Hampe (1881) described *Hookeria subnitens* var. *latior* from the specimen *Puiggari 538*, collected in May 1879 near Apiahy, Brazil. The single specimen is deposited in BM and marked as "Herb. Hampe–1881" and "ex Hb. Geheeb." No additional duplicates were discovered. This specimen is likely to be the only one used in the description of the new variety and is selected as the lectotype.

Original material of *Hookeria entodontella* is deposited in H as well as in S with duplicates. The collection in H is small, whereas those in S are large. The largest of these is designated as the lectotype.

Crossomitrium portoricense was described from a specimen collected in Puerto Rico by Sintensis in November 1885. The type citation notes the specimen to be deposited in the herbarium of Wilhelm and Urban, which was destroyed in World War II. Original material in BM, G, NY, and S mostly agrees with the specimen information cited in the protologue by Müller except for having "Hb. Krug et Urban" noted on the labels. Most of these specimens are small, but the BM specimen is large. It is designated as the lectotype.

Exsiccatal specimen No. *172* in Ule's (1899) *Bryotheca Brasiliensis* is original material of *Hookeria* (*Lepidopilidium*) *longicuspis* that was considered by Müller (1900). Although the collection number, *1030*, is not cited on any of the specimen labels in BM, BR, G, JE, L, and M, the label information matches the locality data from the protologue, including the date. Since Ule's herbarium was deposited in B, the duplicate bearing the *1030* collection number was likely destroyed during the 1 March 1943 bombing of Berlin (Merrill, 1943). A particularly large specimen with numerous sporophytes in G is designated as the lectotype.

Hookeria (*Lepidopilidium*) *tenuiseta* is based on two syntypic specimens collected by Ule (*317* and *468*). Only specimen *Ule 317* was located for this study, and it is designated as the lectotype.

Müller (1900) described *Hookeria gracilifrons* based on the specimen *Ule 1470*, collected from Minas Gerais, Brazil, in March 1892. The specimen deposited in Müller's herbarium at B was destroyed, but duplicates were located in S and US. The specimen in S is larger than the one in US and is designated as the lectotype.

Brotherus described *Hookeria wainioi* from an unnumbered specimen collected in 1885 by Wainio from Minas Gerais, Brazil. Original material is deposited in S, but no material of the type collection was located in Brotherus's herbarium in H. The specimen in S is designated as the lectotype.

Discussion. *Lepidopilidium nitens* has prostrate, moderately to strongly complanate-foliate stems; uniseriate, multicellular gemmae; dioicous sexuality; elongate, smooth to distally papillose setae; and suberect to inclined, obloid-cylindrical capsules. As seen from the list of conspecific synonyms, the species otherwise exhibits considerable plasticity, especially in its lateral leaves. In fact, the majority of the synonyms were originally described for differences in lateral leaf characters, such as color (*L. aureopurpureum* and *L. entodontella*), symmetry (*L. subnitens*), shape and size (*L. entodontella*, *L. laevisetum*, *L. regnellii*, *L. subnitens*, and *L. tenuisetum*), length of the apices (*L. gracilifrons* and *L. longicuspis*), serration of the margins (*L. laevisetum* and *L. wainioi*), length and ornamentation of the costae (*L. subnitens*), and the shape and size of the apical and median leaf cells (*L. gracilifrons* and *L. portoricense*). However, despite the large number of names, few of them were ever applied to specimens other than those for which they were described. Only the name *L. portoricense* has been widely used for specimens outside of a regional context, which is likely the result of it having been illustrated in several major floristic publications.

Brotherus (1924) was the first to recognize that *Lepidopilidium nitens* was polymorphic when he placed the names *L. longicuspis*, *L. subnitens*, and *L. tenuisetum* into synonymy. Although Brotherus also used names that are here considered synonymous to *L. nitens*, he no doubt encountered difficulties in applying them. For example, using strict type concepts, Brotherus assigned three different names to Schiffner's specimens from southeastern Brazil that are close in sequential collection numbers and have identical label information. The name *L. aureopurpureum* was used for No. *1833*; *L. gracilifrons* was given to No. *1834*; and *L. laevisetum* was used for No. *1847*. A re-examination of these three collections found intergradations in the symmetry and shape of the lateral leaves, length of the apices, serration of the upper leaf margins, length of the costae, and shape and length of the apical and median leaf cells. In fact, plants in the same collection, but inhabiting different parts of the substrate, were found to have morphological differences. In addition, lateral leaves examined from near the tips and ends of stems and branches were found to be morphologically dissimilar from those on older sections of the stem. Depending on which lateral leaves are used in making the determination, a single stem can be assigned to at least two different names.

In this revision, approximately 250 specimens of *Lepidopilidium nitens* were examined from throughout the range of the now synonymous names. Due to the observed variability within individual specimens as well as on individual stems none of the previously recognized names are considered tenable at any formal taxonomic rank. However, specimens do seem to correspond to three morphological expressions, albeit with some clinal tendencies.

Key to the Morphological Expressions of *Lepidopilidium nitens*

1a. Plants strongly complanate, stems mostly unbranched; lateral leaves strongly asymmetric and elliptical or ovate; apices acute to short-acuminate; margins entire or serrulate; upper leaf cells hexagonal to short-rhomboidal; costae ending at midleaf or below and not projecting at the tips ..... *L. regnellii* (Ångstr.) W. R. Buck & Ireland

1b. Plants complanate, frequently and irregularly branched; lateral leaves symmetrical or asymmetrical, variously ovate, oblong, or lanceolate; margins serrulate to serrate; upper leaf cells short to elongate-rhomboidal, or fusiform; costae ending at midleaf or above, sometimes projecting at the tips.

2a. Lateral leaves mostly asymmetrical, ovate or oblong; apices abruptly and narrowly short- to long-acuminate, the acumen reaching to 0.4 mm long; upper leaf cells short to elongate-rhomboidal; costae extending to midleaf ..... *L. nitens* (Hornsch.) Broth.

2b. Lateral leaves mostly symmetric or slightly asymmetric, oblong, lanceolate-oblong, or ovate-lanceolate; apices gradually acuminate, narrowing from midleaf; upper leaf cells fusiform; costae ending above midleaf, sometimes with a few weak teeth at back ..... *L. subnitens* (Geh. & Hampe) Broth.

The typical morphological expression of *Lepidopilidium nitens*, including the types of *L. gracilifrons*, *L. portoricense*, *L. tenuisetum*, and *L. wainioi*, has been illustrated in several regional treatments (Bowers, 1994; Buck, 1998; Peralta & Yano, 2006; Allen, 2010). Comparisons of these illustrations indicate some of the variability associated with the lateral leaf shape, length of the apices, leaf margin serration, and length of the costae. This phenotypic variability has been collected from the trunks, twigs, and leaves of trees and shrubs as well as on rotting logs and damp, shaded rocks. The plants are often tinted red, and have complanate, irregularly branched stems and evenly spaced leaves. The lateral leaves are asymmetrical, ovate or oblong, and somewhat concave. The length of the lateral leaf apices vary from abruptly and narrowly short- to long-acuminate, the acumen reaching up to 0.4 mm long. In addition, the leaves are sometimes bordered by a few narrowly elongate cells. The margins are serrate to serrulate above, rarely entire, and the

marginal teeth are occasionally and sporadically bifid, especially noticeable at the apical tips of specimens with long-acuminate apices. The costae extend to midleaf or slightly above and sometimes project at the tips. The apical and median cells are mostly short to elongate-rhomboidal but occasionally can be hexagonal in oblong-ovate leaves or elongate-fusiform in more oblong-lanceolate leaves.

The next morphological expression, centered on the name *Lepidopilidium regnellii*, is considered to be a colonizing growth form that occurs on rotting wood, bases of trees, and on moist, shaded rocks. It contains mostly unpigmented plants that are strongly flattened, have mostly unbranched stems, and bear numerous gemmae. In addition, the leaves are crowded, strongly asymmetric, elliptical or ovate, flat, and have acute to short-acuminate apices; the leaf margins are elimbate, mostly entire to serrulate; the costae are uneven, diverge, and end below midleaf; and the apical and median leaf cells are hexagonal to rhomboidal. Specimens with sporophytes have not been observed. This expression is difficult to separate from the scabrisetum group in *Lepidopilum*, based on *L. scabrisetum*.

At the opposite extreme of the *Lepidopilidium regnellii* expression for variability in *L. nitens* is a more robust and densely foliate expression that occurs on tree branches, twigs, and leaves. It includes the types of *L. aureopurpureum*, *L. entodontella*, *L. longicuspis*, and *L. subnitens*, and has been cited by several authors as *L. laevisetum* (Sehnem, 1979; Robinson, 1986; Buck et al., 2005; Vaz & Costa, 2006; Vaz-Imbassahy & Costa, 2008). The type of *L. laevisetum* (*Glaziou 9235*, BM) has asymmetrical, oblong to ovate leaves (1.4–2 × 0.7–9 mm), costae that do not project at the tips, and rhomboidal leaf cells (20–60 × 12–16 μm). It is a better match for the typical expression of *L. nitens* than the *subnitens* expression. Although *L. laevisetum* was originally described as autoicous, the type is in fact dioicous. The *subnitens* expression is yellow-green to golden in color and sometimes tinted red. The stems are complanate-foliate, frequently and irregularly branched, and have closely spaced, slightly shriveled leaves when dry. The lateral leaves are more or less symmetric, lanceolate to oblong-lanceolate, and have broadly to narrowly long-acuminate apices. Additionally, the leaf margins are serrate to sharply serrate with a few occasional bifid teeth; the costae extend to midleaf or above, are sometimes toothed at back, and often weakly to strongly project at the tips. Lastly, the apical and median leaf cells are mostly fusiform throughout.

Additional specimens examined. BELIZE. **Cayo:** Doyles's Delight, *Allen 15042* (MO), *15095* (MO), *15115* (MO), *15243* (MO); Maya Mtns. above Mukle Bal Tsul ruin, Bladen Nature Reserve, *Whittemore 6230* (MO). **Toledo:** Doyles's Delight, *Allen 15462* (MO), *15457* (MO), *15471B* (MO), *15486B* (MO), *15121* (MO), *15374* (MO), *15424* (MO), *15483* (MO); Columbia River Forest Reserve, *Allen 18826* (MO), *18898* (MO), *18930* (MO), *18950* (MO), *18951* (MO), *18955* (MO, NY), *19014* (MO), *Holst 4335* (MO); Middle Snake Creek basin, Bladen Nature Reserve, *Whittemore 6339* (MO). COSTA RICA. **Alajuela:** Santiago, San Ramón, 1901, *Brenes s.n.* (NY). **Cartago:** W bank of Río Grande de Orosi, above confluence with Río Villegas, *Crosby 8578* (MO). **Puntarenas:** Monteverde, *James 1969-80* (MO). **San José:** vic. Santa Mariá de Dota, *Standley 42431B* (FH, NY). HONDURAS. **Copán:** Copán Ruinas Mpio., *Allen 17729* (MO). NICARAGUA. **Nueva Segovia:** Cerro Mogotón, *Magill 14812* (MO).

CUBA. **Granma:** Falda Sierra Maestra, NE del Puntón del Mate, *Bucher 457* (FH, NY). **Santiago de Cuba:** La Gran Piedra, *Buck 7562* (NY); Loma del Gato, *Hioram 11951* (NY), *Leon et al. 10257* (NY); Loma San Juan, *Hioram 12703* (NY), *13702* (NY); Sevilla Estate, near Santiago, Jiquarito Mtn., Sierra Maestra, *Taylor 492* (NY). DOMINICA. **St. Andrew Parish:** Morne Diablotin, *Elliott 1068* (BM, FH, NY). **St. Joseph Parish:** Morne Couronne, *Elliott 206a* (BM, FH, NY). **St. Patrick Parish:** Morne Trois Pitons, *Elliott 2322* (BM, FH, NY). DOMINICAN REPUBLIC. **La Vega:** Loma Campana, *Allard 18633* (NY), *18778* (NY), *18780* (NY). REPUBLIC OF TRINIDAD AND TOBAGO. **Saint George County:** trail to Morne Bleu from summit of Arima-Blanchisseuse Rd., *Crosby 2110* (MO). PUERTO RICO. **Adjuntas:** Alto de la Bandera, *Britton & Marble 2212* (MO, NY). **Barranquitas:** Monte Torrecilla, *Britton et al. 5693* (NY); upper slopes of La Torrecilla, *Steere 4522* (MO, NY), *4543* (MO), *4562* (MO), *4563* (MO), *4565* (MO), *4567* (MO), *4571* (MO), *4585* (MO), *4645* (MO), *4631* (MO), *4633* (MO), *4906a* (MO). **Cayey:** Twin Mtn. betw. Cayey & Salinas, *Steere 5268* (MO, NY), *5269* (MO). **Guayama:** mtn. betw. Guayama & Cayey, *Britton et al. 6581* (NY). **Jayuua:** Toro Negro State Forest, *Griffin 325* (NY); Santa Rosa, *Pagán 314* (MO); Los Picachos de Jayuya, *Pagán 308* (MO), *311* (MO), *325* (MO); Serro de la Punta, *Steere 6168* (MO, NY); Toro Negro Reservoir, *Steere 6902* (MO, NY). **Lares:** Monte Torrecilla, July 1911, *Hioram s.n.* (NY). **Maricao:** Maricao to Monte Alegrillo, *Britton 2645* (MO, NY, S), *2647* (NY, S); Reserva Forestal Maricao, along Hwy. 120 at Km. 13.8, *Reese 14567* (MO); CCC trail number, *Steere 5555* (MO, NY); summit of high ridge, *Steere 5763* (MO), *5789* (MO), *5800* (MO). **Ponce:** Hac. San Narciso, *Britton & Britton 7308* (NY). **Rio Grande:** El Yunque Trail, *Grout & Steere 60* (NY), *67* (NY), *70* (NY). **Utuado:** Mt. Morales, near Utado, *Britton & Marble 1179* (NY).

BRAZIL. **Bahia:** Mpio. de Ilhéus, cidade de Ilhéus, Mata da Esperança, *Lisbôa de Oliveira 80* (NY). **Minas Gerais:** Caldas, s.d., *Regnell s.n.* (S). **Paraná:** Serra do Mar, Carvalho, *Dusén 8617* (FH, M, S); São José dos Pinhais, Serra do Emboque, *Hatschbach 19658* (NY); Quatro Barras, Morro Mãe Catira, *Kummrow 3049* (NY). **Rio de Janerio:** *Glaziou 7090 p.p.* (BM, NY, S); Corcovado, Dec. 1911, *Herzog s.n.* (JE, S), *12* (JE); *Mosén 157* (H, S); Natl. park Itatiaia bei Itatiaia, an schattigem Gestein im Regenwald beim Wasserfall Veu da Noiva, *Schäfer-Verwimp 9237* (NY); Petropolis, Araras, base da Pedra Maria Comprida, *Sucre 2552* (MO); Novo Friburgo, Feb. 1900, *Ule s.n.* (FH); Parque Nac. Itatiaia along trail to Véu de Noiva & Itaporani,

Vital & Buck 19980 (MO, NY, SP), *20025* (MO, NY). **Rio Grande do Sul:** São Francisco de Paula, Taimbé, *Sehnem 6879* (NY). **São Paulo:** *Hoehne 404* (JE); Apiahy, 1879, *Puiggari 540* (syntype, *Hookeria subnitens*, JE!, L!, S!); Apiahy, July 1880, *Puiggari s.n.* (JE); Apiahy, Aug. 1882, *Puiggari s.n.* (JE); prope Iporange, 1879, *Puiggari 929* (syntype, *Hookeria subnitens*, BM!, H!, JE!); Alto da Serra, *Hoehne 16/a* (JE); Alto da Serra, *Hoehne 37* (JE, M); Bosque da Saúde, *Hoehne s.n.* (M), *48* (JE), *56* (JE), *57* (JE), *64* (JE); Estrada do Vergueiro, *Hoehne 122* (JE, M, S); Estação Biol., Alto da Serra, *Hoehne 673* (JE); Tabapuã, *Hoehne 72* (JE, S), *91* (JE), *315* (JE), *382* (JE); Estação Biol., Alto da Serra, *Gehrt 506* (JE); Serra do Mar bei Paranapiacaba, zwischen São Paulo & Santos, *Schäfer-Verwimp & Verwimp 7141* (NY); prope Rio Grande ad São Paulo Railway, *Schiffner 512*, *688* (BM, M, S); Rio Mambu, Conceição de Itanhaen, *Schiffner 538* (S), *1430* (BM, M); Alto da Serra, *Schiffner 860* (BM, S), *1305* (BM); prope Barra Mansa, distr. Urbis Itapecirica, *Schiffner 1453* (BM); prope São Bernardo haud procul ab urbe São Paulo, *Schiffner 1833* (BM), *1834* (BM), *1847* (BM); Serra do Cayazique, prope Santos, *Schiffner 1856* (BM); Pindamonhagaba Co., ca. 2 km NE from Pico de Itapeva, *Vital DV-1940* (NY); Mpio. Juquitiba, *Vital 7143* (MO). FRENCH GUIANA. **Cayenne:** Sommet Tabulaire, *Cremers 6787* (U), *6791* (U). **Saint-Laurent-du-Maroni:** Canton de Maripasoula, *Buck 25571* (NY), *25572* (NY), *25582* (NY), *25622* (NY), *25637* (MO, NY), *25730* (NY), *25747* (NY), *25770* (NY), *25774* (NY); Montagne de l'Inini, *Onraedt 8979* (U), *8980* (U), *8990* (U), *8995* (U), *8996* (U), *9004* (U), *9059* (U), *9079* (U), *9082* (U), *9171* (U), *9227* (U), *9239* (U), *9245* (U), *9246* (U), *9249* (U), *9265* (U). **Saül:** Mont Galbao, *Buck 33253* (MO, NY), *33303* (NY), *Cremers 4049* (U), *4050* (U), *4098* (U), *4104* (U), *4149* (U), *6098* (U). GUYANA. **Cuyuni-Mazaruni:** upper Mazaruni River, Karowtipu Mtn., *Boom & Gopaul 7473* (NY); trail from Kamarang River to Pwipwi Mtn., *Gradstein 5717* (U). **Essequibo Islands-West Demerara:** Essequibo River, Moraballi Creek near Bartica, *Richards 309* (NY), *321* (NY, U). **Potaro-Siparuni:** Kaieteur Falls Natl. Park, *Newton 3422* (U); Paramakatoi, Para Mtn., *Newton 3534* (U), *3545* (U), *3551* (U). PARAGUAY. **Caazapá:** Estancia Golondrina, Reserva Privada Ypeti, *Zardini & Hellman 49038* (MO). SURINAME. **Brokopondo:** Brownsberg Massif, *Allen 19408* (MO); Brownsberg Nature Park, *Florschütz-de Waard & Florschütz 4622* (U), *4702* (U), *4722* (U), *4752* (U), *4759* (U), *Florschütz-de Waard & Zielman 5032* (U). **Sipaliwini:** Tafelberg, W rim of Arrowhead Basin, *Allen 23684* (MO), *23690* (MO, NY); Tafelberg, trail from Augustus Creek to North Ridge, *Allen 20578* (MO), *20630* (MO), *23339* (MO), *23348* (MO), *23363* (MO); Eilerts De Haan Gebergte, *Allen 25502* (U), *25528* (MO); Emmaketen, *BW 5655A* (U); Helling Ebbatop, van Asch van Wijcksgebergte, *Florschütz-de Waard & Florschütz 1486* (U), *1490* (U); Lely Mtns., *Florschütz-de Waard & Florschütz 4811* (U), *4871* (U), *Lindeman et al. 61b* (U), *506* (U); Bakhuis Mtns., in montibus Bakhuis inter flum. Kabalebo et Coppename Sinistrum, *Florschütz-de Waard & Maas 2870* (U), *2935* (U); Nassau Mtns., *Lindeman 7040* (U). VENEZUELA. **Aragua:** Parque Nac. Henri Pittier, bosque de Rancho Grande, *Agostini & Cobo 197* (MO, NY, U), *199* (MO); Distr. Girardot, Parque Nac. Henri Pittier (Rancho Grande), La Cumbre de Rancho Grande trail, *Davidse et al. 16667C* (MO), *16693* (MO); Parque Nac. Henri Pittier, Portachuelo, *Hokche et al. 810* (U), *Luteyn 8226* (U); Parque Nac. Henri Pittier, cumber Rancho Grande, *Morales 1737* (MO); Nebelwald von Rancho Grande, *Mägdefrau 350* (M), *372* (M, U); Maracay, Choroní, *Mägdefrau 476* (M, U), *487* (M, U), *497* (M); Rancho Grande, 17 Jan. 1971, *Ojasti s.n.* (MO, U). **Distrito Federal:** 6 km W of El Junquito on rd. betw. Caracas & Colonia Tovar, *Nee & Whalen 16865* (NY), *16868* (NY). **Falcón:** Sierra de San Luis, vía Coro-Uria, *Griffin & López-Figueiras PV-1614* (NY). **Lara:** Distr. Palavecino, selva dublada, Fila de Terepaima, entre el Alto de Chuparral y la Loma Redonda, *Steyermark et al. 103395* (FH, MO, U, US). **Miranda:** Parque Nac. Macarao, el junquito, *Morales 1441* (MO); Parque Nac. Guatopo, NE of hwy., 18 km NNW of Altagracia de Orituco, *Nee 17910* (NY), *17914* (NY), *17919* (NY), *17921* (NY), *17922* (NY); Cerros del Bachiller, *Steyermark & Davidse 116605* (MO), *116606* (MO), *116607* (MO), *116686* (MO), *116709A* (MO), *116838A* (MO). **Sucre:** Peninsula de Paria, Cerro de Río Arriba, *Steyermark & Rabe 96207* (US).

Excluded Taxa

Lepidopilum caudicaule Müll. Hal., Hedwigia 39: 272. 1900. *Lepidopilidium caudicaule* (Müll. Hal.) Broth., Nat. Pflanzenfam. [Engler & Prantl] 1(3): 944. 1907. TYPE CITATION: Brasilia, Rio de Janeiro, Serra dos Orgãos, ad rupes Serrae, Dec. 1891, *E. Ule*, Coll. No. *1249*. TYPE: Brazil. Minas Gerais, Serra dos Orgaos, an Falsom in der Serra, Dec. 1891, [*E.*] *Ule 1249* (types, H!, US!).

Müller (1900) described *Lepidopilum caudicaule* based on Ule's specimen No. *1249*, which lacked sporophytes. Although the name was originally placed in *Lepidopilum*, Müller suggested *Lepidopilidium* as a possible alternative genus. The name has since been transferred to *Lepidopilidium* (Brotherus, 1907), although the original placement has also been maintained (Sehnem, 1979; Vaz & Costa, 2006; Vaz-Imbassahy & Costa, 2008). Sehnem (1979) based his decision to include *Lepidopilum caudicaule* in *Lepidopilum* on one of his own specimens that was gametophytically similar to the type description, was collected from a habitat similar to that cited in the protologue, and had sporophytes. Vaz-Imbassahy and Costa (2008) corroborated Sehnem's specimen with a type in R that they illustrate as having sporophytes, despite this being contrary to the type description. Type specimens in H and US lack sporophytes.

Lepidopilum pallidonitens (Müll. Hal.) Paris, Index Bryol. Suppl. 223. 1900. TYPE: Bolivia. Cochabamba, prope Choquecamata, June 1889, *Germain s.n.* (type, NY not seen).

Lepidopilum plebejum Müll. Hal., Hedwigia 39: 272. 1900. *Lepidopilidium plebejum* (Müll. Hal.) Sehnem, Pesquisas, Bot. 33: 32. 1979, syn. nov. TYPE CITATION: Brasilia, Sa. Catharina, Tubarão, in lingo putrido sylvestri, Oct. 1889, *E. Ule*, Coll. No. *768*; colonia Blumenau, ad truncum arboris sylvestris ad rivulum Ribeirão-fresco, Sep. 1888, idem, Coll. No. *473*. TYPE: [Brazil.] Estado de Santa Catarina. Num tronco no mato do Ribeirão-fresco perto de Blumenau. Sep.

1888, [*E.*] *Ule 473* (lectotype, designated here, PC!; isolectotype, H!).

Müller (1900) originally described *Lepidopilum plebejum* Müll. Hal. from two specimens that lacked sporophytes. A detailed description of the sporophyte was later provided by Sehnem (1979) based on his own specimen that had been collected in a habitat similar to that described in the protologue. Sehnem described the sporophytes as having distally papillose, 3-mm setae, with erect, 1-mm-long capsules and a hookeriaceous-type peristome. These characters led him to transfer *Lepidopilum plebejum* to *Lepidopilidium*. However, Sehnem's sporophyte description and specimen, *Sehnem 6491* (NY), belongs to *Lepidopilidium brevisteum*. Despite the lack of sporophytes in the type specimen, *Lepidopilum plebejum* is better placed in synonymy with *Lepidopilum pallidonitens* (Müll. Hal.) Paris. The specimen *Ule 473* is selected as the lectotype. It has strongly complanate, oblong-ovate, 2- to 3-mm-long leaves, broadly acuminate and apiculate apices, serrulate margins, and long-fusiform laminal cells that are shorter near the leaf apices and correspond to that of *L. pallidonitens*.

Hookeriopsis pallidifolia (Mitt.) Geh. & Herzog, Biblioth. Bot. 73: 59. 1910. *Hookeria pallidifolia* Mitt., in J. C. Melliss, St. Helena 362. 1875. *Lepidopilidium pallidifolium* (Mitt.) Vaz-Imbassahy & D. P. Costa, Nova Hedwigia 88: 467. 2009. TYPE CITATION: amongst the grass on the high land; a bright-green moss. *Dr. Hooker*, Melliss; also in *Herb. Turner* the specimen marked East Indies, *J. D.* (*Dickson*). TYPE: [United Kingdom, overseas territory]. St. Helena Island, s.d., *Hooker 427* (lectotype, designated by Vaz-Imbassahy & Costa, 2009: 467, NY!; isolectotype, BM!).

Mitten (1875) described *Hookeria pallidifolia* from a specimen collected by J. D. Hooker on St. Helena Island and another obtained by J. D. Dickson that was labeled East Indies. The original description, which was later repeated by Geheeb and Herzog (1910), remains the only detailed account of *H. pallidifolia*. The species is characterized as small, loosely tufted, and irregularly branched; having slightly asymmetrical and concave, oblong-lanceolate leaves; acute apices; inflexed, entire to crenulate margins; short and inconspicuously forked costae; narrowly elongate leaf cells; smooth, reddish setae; inclined, oval capsules; and a hookeriaceous-type peristome. *Hookeria pallidifolia* was transferred to *Hookeriopsis* s.l. (*Brymela* Crosby & B. H. Allen, *Thamniopsis*, and *Trachyxiphium* W. R. Buck) by Geheeb and Herzog (1910) and to *Lepidopilidium* by Vaz-Imbassahy and Costa (2009). However, the species belongs in *Hookeriopsis* s. str. as evident by a few critical characters. First, the stems of *H. pallidifolia* lack a developed hyalodermis in cross-section. Although *Brymela*, *Hookeriopsis* s. str., and *Trachyxiphium* also lack a stem hyalodermis, both *Lepidopilidium* and *Thamniopsis* have a moderately to well-developed hyalodermis. Next, the leaf margins of *H. pallidifolia* are entire to crenulate. While the leaf margins of both *Thamniopsis* and *Trachyxiphium* bear swollen, often bifid teeth, those of *Brymela*, *Hookeriopsis* s. str., and *Lepidopilidium* are mostly simple or have entire margins. Furthermore, unlike the prominent costae found in *Brymela* and *Lepidopilidium*, the costae in *Hookeriopsis* s. str. are short, weakly prominent, terminate below the middle of the leaf, and do not project at the tips. Lastly, the stomata of *H. pallidifolia* are rudimentary with four or five subsidiary cells, unlike those of *Lepidopilidium*, which only have two subsidiary cells.

Some confusion surrounds the distribution of *Hookeriopsis pallidifolia* based on the specimen cited in Mitten's (1875) protologue. Dickson obtained his East Indies specimen from a returning trade vessel that likely noted only the last destination in its voyage. According to Mitten (1875: 360), Dickson's East Indies specimens are likely to have been collected on St. Helena Island.

Hookeriopsis crispifolia (W. R. Buck & M. Wigginton) J. J. Atwood, comb. nov. Basionym: *Lepidopilidium crispifolium* W. R. Buck & M. Wigginton, in Wigginton & Buck, J. Bryol. 35: 270. 2013. TYPE CITATION: St. Helena. Diana's Peak National Park, compartment Actaeo 09, wet rocks by stream in deep valley, shaded under ferns, growing with *Riccardia* Gray sp. and *Fissidens elegans* Brid., 744 m, 30L/TH/107323, 5°42′W, 15°58′S, 25 Oct. 2005, *Wigginton 05/237*. TYPE: [United Kingdom, overseas territory.] St. Helena. Diana's Peak Natl. Park, Cuckold's Peak, Actaeo 09, wet rocks by stream, shaded under ferns in deep valley; with *Riccardia* spp. and *Fissidens elegans*, 744 m, 30L/TH/107323, 5°42′W, 15°58′S, 25 Oct. 2005, *Wigginton 05/237* (holotype, E not seen; isotype, NY!).

Lepidopilidium crispifolium was described from St. Helena Island based on specimens having strongly contorted, twisted leaves; plane leaf margins; short, double costae that are one third the leaf length; autoicous sexuality; and long, smooth setae. It also deviates from *Lepidopilidium* in a few critical characteristics. *Lepidopilidium crispifolium* lacks both

a complanate habit and a stem hyalodermis. Furthermore, the stomata are rudimentary with four or five subsidiary cells. Although *L. crispifolium* was described as lacking an annulus, a persistent annulus of two or three rows of subquadrate, differentiated cells, with one to three riblike thickenings on the inner cell walls, was found attached to an operculum in *Wigginton 05/549* (NY). This combination of characters indicates a closer relationship to *Brymela* and *Hookeriopsis* s. str. rather than *Lepidopilidium*. *Brymela* and *Hookeriopsis* s. str. differ mainly in the length of their costae: *Brymela* has thick, long costae, whereas *Hookeriopsis* s. str. has short, indistinct costae. Since *L. crispifolium* also has short, indistinct costae, it is better placed with *H. pallidifolia* in *Hookeriopsis* s. str. The two species represent the first reports of *Hookeriopsis* s. str. from outside of the Caribbean.

Thamniopsis chenagonii (Renauld & Cardot) J. J. Atwood, comb. nov. Basionym: *Lepidopilum chenagonii* Renauld & Cardot, Suppl. Prodr. Fl. Bryol. Madagascar 80. 1909. *Lepidopilidium chenagonii* (Renauld & Cardot) Renauld & Cardot, in Grandidier, Hist. Phys. Madagascar, Mousses 415. 1915. TYPE CITATION: Madagascar: Montagne d'Ambre près Diego Suarez, *Chenagon*, 1890. TYPE: Madagascar. Diego Suarez, 1890, *Chenagon s.n.* (lectotype, designated here, PC!; isotype, PC!).

Thamniopsis chenagonii is a yellowish green, slender species with weakly complanate-foliate stems and erect branches. In cross-section, the stems have a well-developed hyalodermis, two or three rows of small, thick-walled subepidermal cells, and enlarged cortical cells. The dorsal, ventral, and lateral leaves are somewhat contorted when dry, short, asymmetrical, and oblong-ovate, and have acute to broadly acuminate apices. In addition, the leaves have rhomboidal apical and median cells, swollen teeth along the upper leaf margins, and costae that extend to above the midleaf, prominently project at the tips, and are toothed along the back. The setae of *T. chenagonii* are reddish yellow, elongate (5–7 mm), flexuous, and smooth. The capsules are horizontal to pendent, short, and ovoid. The exothecial cells are collenchymatous, and the stomata are scattered on the neck, rudimentary, and composed of four subsidiary cells. The presence of the rudimentary stomata, consistently inflated marginal teeth, and costae with prominent serrations exclude this species from *Lepidopilidium*. The name is better placed in the more widely circumscribed genus *Thamniopsis*. Duplicates of the type collection are deposited at PC. The largest of these is designated as the lectotype.

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THE EVOLUTION OF VIOLACEAE FROM AN ANATOMICAL AND MORPHOLOGICAL PERSPECTIVE[1]

Saúl E. Hoyos-Gómez[2]

ABSTRACT

Recent work suggests that *Fusispermum* Cuatrec. and *Rinorea* Aubl. form small clades that are sister to the rest of the Violaceae and that the Goupiaceae is sister to the Violaceae. However, little is known about the morphology and anatomy of these phylogenetically critical groups. In this paper I present aspects of the morphology and anatomy of stem, node, leaf, flower, and seed of three species of *Fusispermum* and seven species of *Rinorea*, as well as *Goupia glabra* Aubl. (Goupiaceae), which is the outgroup. Placing this variation in the context of hypothesized phylogenetic relationships, I found *Fusispermum* to have unique pentalacunar nodes, heterogeneous pith, and elongated seeds, while *Goupia* Aubl. has a unique 5-carpellate gynoecium with marginal styles and a tegmen with U-shaped thickenings. Furthermore, variation in androecium and nectary links the distinctive androecium so common in the Violaceae with more conventional structures found in other taxa of the parietal placentation group of Malpighiales. Strengthening our basic knowledge of anatomy and morphology in these groups is an essential prerequisite for understanding the evolution and diversification not only of Violaceae but of Malpighiales as a whole.

Key words: Anatomy, flower, *Fusispermum*, *Goupia*, Goupiaceae, leaf and seed morphology, Malpighiales, *Rinorea*, stem, Violaceae.

The violet family (Violaceae) is predominantly tropical in distribution and contains 23 genera and more than 900 species (Feng, 2005; Tokuoka, 2008). The family is supported as monophyletic (Feng, 2005; Tokuoka, 2008), although phylogenetic relationships within it are still unclear (Feng, 2005; Tokuoka, 2008). The Violaceae encompasses a great diversity of vegetative and floral variation. Habits may vary from herbs to lianas to trees, with flowers ranging from zygomorphic (monosymmetric) and strongly spurred to actinomorphic (polysymmetric) and unspurred. Anther variation is considerable, and there is variation in shape and structure of the androecium in the Neotropical *Rinorea* Aubl., especially in the connective scales. The fruits range from an indehiscent nut or fleshy berry to loculicidal capsules, sometimes explosively dehiscent; the seeds are small to noticeably large and may be winged or carunculate (Hekking, 1988).

Recent studies (Wahlert et al., 2014) have changed our understanding of the phylogeny of Violaceae by elucidating unsuspected or lesser-known groups that are prime candidates for the study of morphology and anatomy. Within the Violaceae, clades for *Fusispermum* Cuatrec. and *Rinorea apiculata* Hekking (Wahlert & Ballard, 2012) are strongly supported as being successive sisters to the rest of the family (Fig. 1). The uncommon *Fusispermum* is a Neotropical genus with three species, ranging from Costa Rica to Peru. In contrast, *Rinorea* is a common pantropical genus with 49 species in the Neotropics (Wahlert & Ballard, 2012).

Another development that affects our understanding of the Violaceae is their phylogenetic association with the Goupiaceae, which contains *Goupia* Aubl. with two species, one from Central America (*G. guatemalensis* Lundell), and the other (*G. glabra* Aubl.) from northern South America to the north of the Amazon basin. The phylogenetic position of the Goupiaceae has long been poorly understood. *Goupia* was included in the Celastraceae by Bentham and Hooker (1862) as the subfamily Goupioideae, a view later accepted by Cronquist (1981). The genus has been recognized as its own family (e.g., Simmons et al., 2001). However, *Goupia* has not previously been associated with the Violaceae or with any other family

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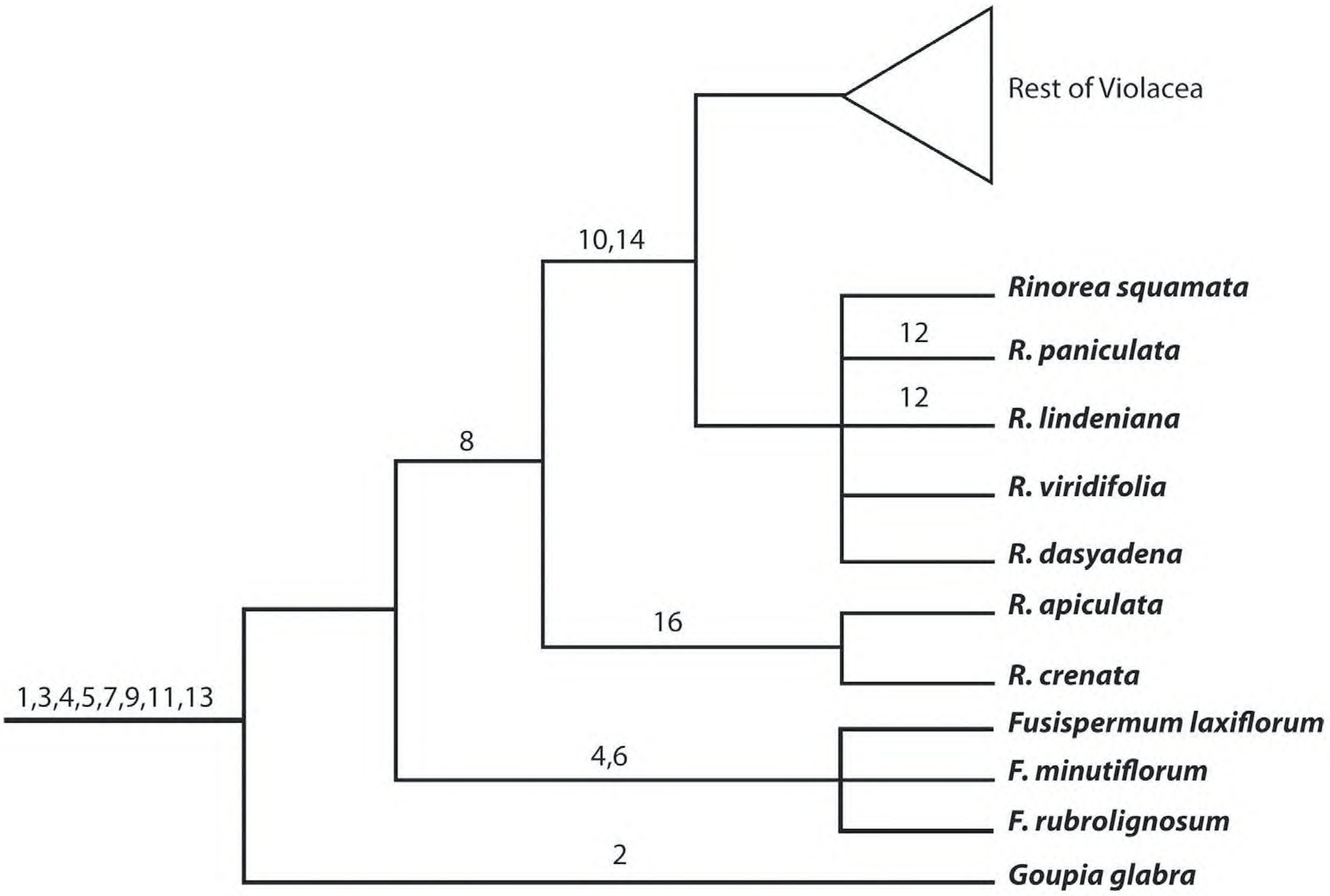


Figure 1. Summary tree diagram for relationships between *Fusispermum* Cuatrec. and *Rinorea* Aubl. (Violaceae) and the outgroup exemplar *Goupia glabra* Aubl. (Goupiaceae). Morphological and anatomical character states useful for understanding relationships among these taxa are mapped on the tree. Those character states found at the stem of this tree (1, 3, 4, 5, 7, 9, 11, 13, 15; see Table 2) may be found in other members of the parietal placenta clade. Mapped characters include (1) cylindrical stem, (2) fluted stem, (3) homogeneous pith, (4) heterogeneous pith, (5) trilacunar nodal anatomy, (6) pentalacunar nodal anatomy, (7) nectary disc, (8) gland adnate to filament, (9) connective scale apical, (10) connective scale basal, (11) style straight, (12) style curved, (13) seed with exotegmen with sclereids (one layer), (14) seed with exotegmen with fibers (four to six layers), (15) stigma area not expanded, and (16) stigma area flared outward.

in the parietal placentation group within the Malpighiales. Placentation in the Goupiaceae has been distinguished as basal-axile (Stevens, 2012).

Simmons et al. (2001), in their study of the relationships of the Celastraceae, first suggested that its subfamily Goupioideae belonged to the Malpighiales. Feng (2005) and Wurdack and Davis (2009) placed it, as the Goupiaceae, as sister to the Violaceae, and both analyses find strong support for this position. The Goupiaceae are part of a clade that includes the Achariaceae, Lacistemataceae, Passifloraceae, and Salicaceae. *Goupia* remains the only taxon in this group that has axile placentation, with all the others presenting parietal placentation (Wurdack & Davis, 2009).

Few comprehensive studies have been published on the Violaceae, and the morphology of the family, particularly *Fusispermum* species and the *Rinorea apiculata* clade, is largely unknown (Wahlert & Ballard, 2012). Even less is known about floral evolution and development (but cf. Arnal, 1945; Feng, 2005). Most studies have focused on *Viola* L., a genus widely distributed across both hemispheres. But *Viola* is atypical in the family in being largely herbaceous, with derived character states, and of largely temperate distribution (Metcalfe & Chalk, 1972; Corner, 1976; Feng, 2005). In contrast, the majority of the family are woody and tropical taxa, so extrapolation from the derived states in *Viola* to the other genera in the family may be inappropriate or misleading.

Metcalfe and Chalk (1972) summarized what little is known of the vegetative anatomy of *Goupia*, *Fusispermum*, and *Rinorea*. For seeds, Hekking (1984) briefly described the external morphology of the seeds of *Fusispermum*. Corner (1976) investigated the seed anatomy of *Viola*, *Hybanthus* Jacq., and two species of Malesian *Rinorea*. The broadest survey of seed anatomy of Violaceae is by Plisko (1992), but only 14 species were studied there. The seed anatomy of *G. glabra* was described by Melikian and Sarinov (2000; but see below). The seed anatomy of *Fusispermum* and the *R. apiculata* group has not been previously described.

In an attempt to develop a better understanding of the evolutionary history of Violaceae, I examine here the stem, leaf, flower, and seed of three species of *Fusispermum*, of the *Rinorea apiculata* clade (*R. apiculata* and *R. crenata* S. F. Blake), and *Rinorea* s. str. (five species considered), as well the outgroup exemplar *Goupia glabra* (Goupiaceae). For these taxa, I describe anatomical and morphological characters of the stem, node, leaf, androecium, gynoecium, and seed anatomy and morphology using scanning electron microscopy (SEM) and light microscopy (LM). I then place this information in the context of proposed phylogenetic relationships among malpighiacous taxa in the Violaceae and Goupiaceae.

Materials and Methods

During the summer of 2010 several field trips were made to Central and South America to obtain plant material. In Bolivia, collection and observations were made in Departamento de la Paz, Provincia Franz Tamayo, Parque Nacional Madidi, Laguna Chalalan, 14°25′39.49″S, 67°54′57.68″W, at an altitude of 350 m. In Colombia, our collection site was Antioquia, Municipio de San Luis, Cañon de Río Claro, Reserva Natural, 05°53′N 074°39′W, 350 m. In Costa Rica, exemplars were taken from Puntarenas, Osa, Sierpe, Reserva Forestal Golfo Dulce, Estación Biológica Los Charcos de Osa, 08°40′18″N 83°30′17″W, 70 m. In Peru, field work occurred in Distrito de Loreto, Provincia de Maynas, carretera Bella Vista–Mazán, 3°31′41.9S, 73°05′45.8W, 86 m. Voucher specimens (cf. Table 1) of the newly collected material were deposited in Costa Rica (INB), Bolivia (LPB), Peru (USM), Colombia (COL, HUA, JAUM), and in the United States (MO). When additional material was needed, I used collections from the Missouri Botanical Garden (MO). No material of *Rinorea oraria* Steyerm. & A. Fernández, which is the third known member of the *R. apiculata* group, was available.

In the field, stem sections, leaves, flowers, and fruits were preserved in 70% ethanol. Leaves from herbarium specimens were used to complete the taxonomic sampling (Table 1). Dried leaves were rehydrated by boiling in water with a drop of detergent before examination. Cross sections of leaves and petioles were handmade, using a razor blade. Microtome sections of seeds were also made. Cresyl violet acetate (CVA) was used for tissue staining, and a 20% calcium chloride solution ($CaCl_2$; Ogburn & Edwards, 2009) was used as a mounting solution (Keating, 1996, 2000). A Canon Power Shot A640 camera (Canon, Tokyo, Japan) coupled to an Olympus BX40 microscope (Olympus, Tokyo, Japan) was used to record all light microscopic images. For SEM imagery, plant material was dehydrated in an increasing ethanol series and then critical-point dried (using an EMITech-K 850 critical-point dryer [EMITech Inc., Fall River, Massachusetts, U.S.A.]). The samples were affixed to supports using carbon adhesive tape and were gold-coated (using a Denton vacuum sputter coater [Denton, Moorestown, New Jersey, U.S.A.]) and were examined using a JEOL Neoscope JCM 5000 SEM (JEOL, Tokyo, Japan). The SEM was used to study cuticle and stomatal microdetail, as well as pollen, fruit, and flower ultrastructure.

Results

YOUNG STEMS

All species studied had lenticels on the stem and a cork cambium that developed early and was superficial in position. A band of collenchyma tissue ca. five to 10 cells broad was observed in the outer cortex. *Goupia glabra* (Goupiaceae) lacked crystals and druses in the collenchyma; however, all Violaceae studied had rhombic crystals and druses of calcium oxalate there. No sclereids or other idioblastic cells were observed in the stem cortex. The stem vasculature of all species studied was eustelic. The central vascular cylinder was noted as more or less circular in transverse section, except in *G. glabra*, where the vascular cylinder was fluted (Fig. 2A). The outermost phloem was completely surrounded by a band of pericyclic fibers. Stem pith was well developed in all species and was usually made up of small, unlignified cells that were more or less similar in size. In *G. glabra*, pith cells were ca. 20–50 μm in diameter (Fig. 2A); in all *Rinorea* species studied, pith cells were 15–40 μm across; in all species the walls of the pith cells were fairly thin, ca. 4–5 μm across (Fig. 2B, C, *R. crenata, R. paniculata* (Mart.) Kuntze, respectively). In contrast, in *Fusispermum laxiflorum* Hekking (Fig. 2D), *F. minutiflorum* Cuatrec., and *F. rubrolignosum* Cuatrec., the pith cells were larger and variable in size, 50–200 μm in diameter; the cell walls were thinner, ca. 2–3 μm across. Pith in *Fusispermum* can be considered heterogeneous in contrast to the more homogeneous condition confirmed in both *Goupia* and *Rinorea* exemplars.

STEM NODES

Goupia glabra (Fig. 2E) and *Rinorea crenata* (Fig. 2F), as did all *Rinorea* exemplars, had three-trace, trilacunar nodes. However, *Fusispermum laxiflorum*

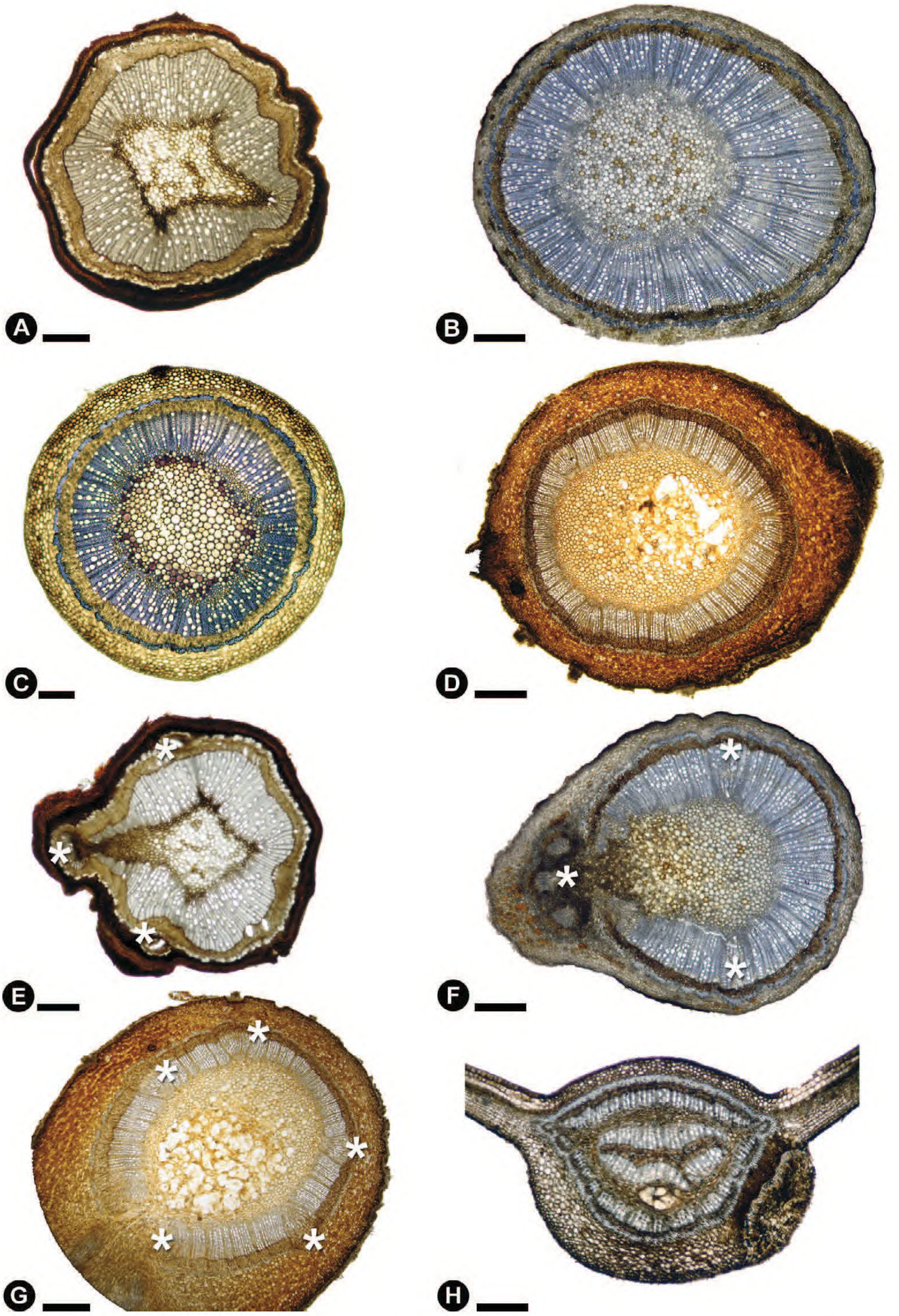


Figure 2. Light microscopic images of stem and leaf blade sections among representative *Rinorea* Aubl. and *Fusispermum* Cuatrec. (Violaceae) and *Goupia* Aubl. (Goupiaceae, outgroup). —A. Transverse stem section, *G. glabra* Aubl. (*Thomas 3992*). —B. Transverse stem section, *R. crenata* S. F. Blake (*S. E. Hoyos-Gómez 1115*). —C. Transverse stem section, *R. paniculata* (Mart.) Kuntze (*S. E. Hoyos-Gómez 1110*). —D. Transverse stem section, *F. laxiflorum* Hekking (*S. E. Hoyos-Gómez 1119*). —E. Internodal stem anatomy, *G. glabra* (*Thomas 3992*). —F. Internodal stem anatomy, *R. crenata* (*S. E. Hoyos-Gómez 1115*). —G. Internodal stem anatomy, *F. laxiflorum* (*S. E. Hoyos-Gómez 1119*). —H. Leaf blade midrib, *F. laxiflorum* (*S. E. Hoyos-Gómez*

(Fig. 2G), *F. minutiflorum*, and *F. rubrolignosum* had pentalacunar nodes, with the vascular traces widely spaced from the central cylinder. In all the species the stipules were vascularized from the lateral traces.

LEAVES

In transverse sections taken at the midpoint of the petiole, peripheral collenchyma tissue was found in all species exemplars. The cells were tanniniferous and contained rhombic crystals and druses, except in *Goupia glabra* (Fig. 3A), which lacked rhombic crystals but did have druses. In all sampled species except *Rinorea apiculata* (Fig. 3B), the vascular tissue formed a more or less closed and somewhat dorsiventrally flattened cylinder with phloem on the outside, xylem on the inside, and the vascular cylinder surrounded by fibers. In *R. apiculata* the main petiole bundle was arcuate, and even there the edges of the arc were strongly incurved. The petiole pith was usually unlignified, but there were dispersed fibers in the pith of the petioles in *G. glabra* (Fig. 3A). All the examined species in *Fusispermum* and *Rinorea* had druses in the pith cells, but *G. glabra* did not. As was seen in the stem cross section, *F. laxiflorum* had much larger and thinner-walled pith cells (Fig. 3C, D) than those of the other examined species.

There were rib traces on either side of the main petiole bundle, usually in adaxial to lateral positions; these traces consisted of arcuate bands of vascular tissue accompanied by a few fibers, especially adjacent to the phloem. There were four rib traces, two on each side of the main bundle, typically seen in *Rinorea apiculata* (Fig. 3B), *R. crenata*, and *R. paniculata*. However, in *R. viridifolia* Rusby, *R. dasyadena* A. Robyns, and *R. squamata* S. F. Blake, only two rib traces were present, one on each side, while in all four specimens of *R. lindeniana* (Tul.) Kuntze (Fig. 3E) examined there were three adaxial rib traces, two on one side and one on the other side above the vascular cylinder.

Fusispermum laxiflorum (Fig. 3C, D), *F. minutiflorum*, and *F. rubrolignosum* have a rather complex petiole vasculature. At the very base of the petiole, immediately after traces depart to the stipules, the vasculature consists of a larger central vascular cylinder and two lateral cylinders on either side, the latter representing the lateral traces. The lateral and central bundles merge and reorganize, becoming broadly incurved and arcuate with a pair of adaxial vascular strands. Finally, toward the apex of the petiole leading into the blade, the vasculature became a more or less complete cylinder surrounding a smaller elliptical cylinder of xylem with phloem on the inside; in addition, there are two small lateral wing bundles on either side of the central cylinder.

In all the *Fusispermum* and *Rinorea* species studied, the leaves were dorsiventrally oriented, with scattered uniseriate hairs on both sides but principally on the abaxial side; *Goupia glabra* had scarce uniseriate hairs on the abaxial side only. Leaf blades varied in thickness from the thickest, ca. 270 μm, in *F. laxiflorum* (Fig. 3F), to ca. 190 μm in *G. glabra* and ca. 150–180 μm among the seven sampled species of *Rinorea* (Fig. 3G, H, *R. apiculata* and *R. squamata*, respectively). In both *G. glabra* and *F. laxiflorum* (Fig. 3F) there was well-defined palisade tissue: with two palisade layers seen in the former and two to three layers in the latter. However, in the seven *Rinorea* species sampled, the palisade tissue was irregularly organized as two to four layers and was not strongly differentiated from the rest of the mesophyll (Fig. 3G, H, *R. apiculata* and *R. squamata*, respectively). No sclereids or other idioblastic cells were observed in either *Fusispermum* or *Rinorea* exemplars. There was some chlorophyllous tissue on the adaxial side of the midrib among the seven species of *Rinorea* but not in *F. laxiflorum* or *G. glabra*. Otherwise the midrib was transcurrent, and smaller veins were embedded.

The vascular tissue in the midrib of the 11 species studied consisted of two arcuate bands of fibers surrounding the vascular tissue. In *Goupia glabra* and *Fusispermum laxiflorum* alone there were also fibers in the medullary tissue of the bundle, and in *F. laxiflorum* they were made up of thin-walled cells like those of the stem pith. In *F. laxiflorum* there were two additional bands of vascular tissue in the midrib that were continuous with the elliptical central cylinder in the petiole.

All 11 species studied had hypostomatic leaves and anomocytic stomata, the stomata being ca. 16–20 μm long. In the seven species of *Rinorea* there were usually three epidermal cells that were directly adjacent to the guard cells, although these adjacent cells were not otherwise distinguishable from the surrounding epidermis. This differed in other taxa in that *Goupia glabra* had four to five epidermal cells adjacent to the stomata, and in *Fusispermum laxiflorum* there were three to five cells directly abutting the stomata.

←

1119). Scale bars in A, D, E, G = 500 μm; scale bars in B, C, F = 350 μm; scale bar in H = 700 μm. Asterisks indicate nodes in E–G. All collections are at MO.

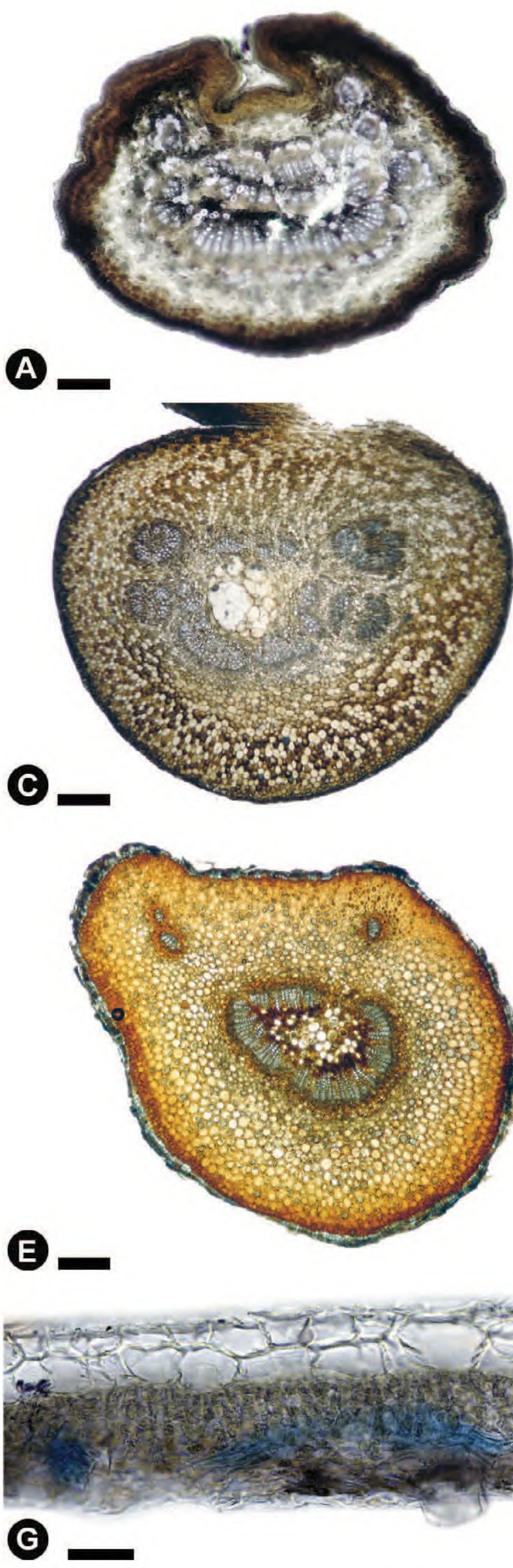


Figure 3. Light microscopic images of leaf petiole and blade sections among representative *Rinorea* Aubl. and *Fusispermum* Cuatrec. (Violaceae) and *Goupia* Aubl. (Goupiaceae, outgroup). —A. Transverse petiole section, *G. glabra* Aubl. (*Thomas 3992*). —B. Transverse petiole section, *R. apiculata* Hekking (*F. Hurtado 2964*). —C, D. Transverse petiole section, *F. laxiflorum* Hekking (*S. E. Hoyos-Gómez 1119*). —E. Hand section of leaf blade, *R. lindeniana* (Tul.) Kuntze (*S. E. Hoyos-Gómez 1135*). —F. Hand section of leaf blade, *F. laxiflorum* (*S. E. Hoyos-Gómez 1119*). —G. Hand section of leaf blade, *R. apiculata* (*P. Nuñez 6026*). —H. Hand section of leaf blade, *R. squamata* S. F. Blake (*S. E. Hoyos-Gómez 1114*). Scale bars in A = 150 μm; B, E = 200 μm; C, D = 300 μm; F–H = 50 μm. All collections are at MO.

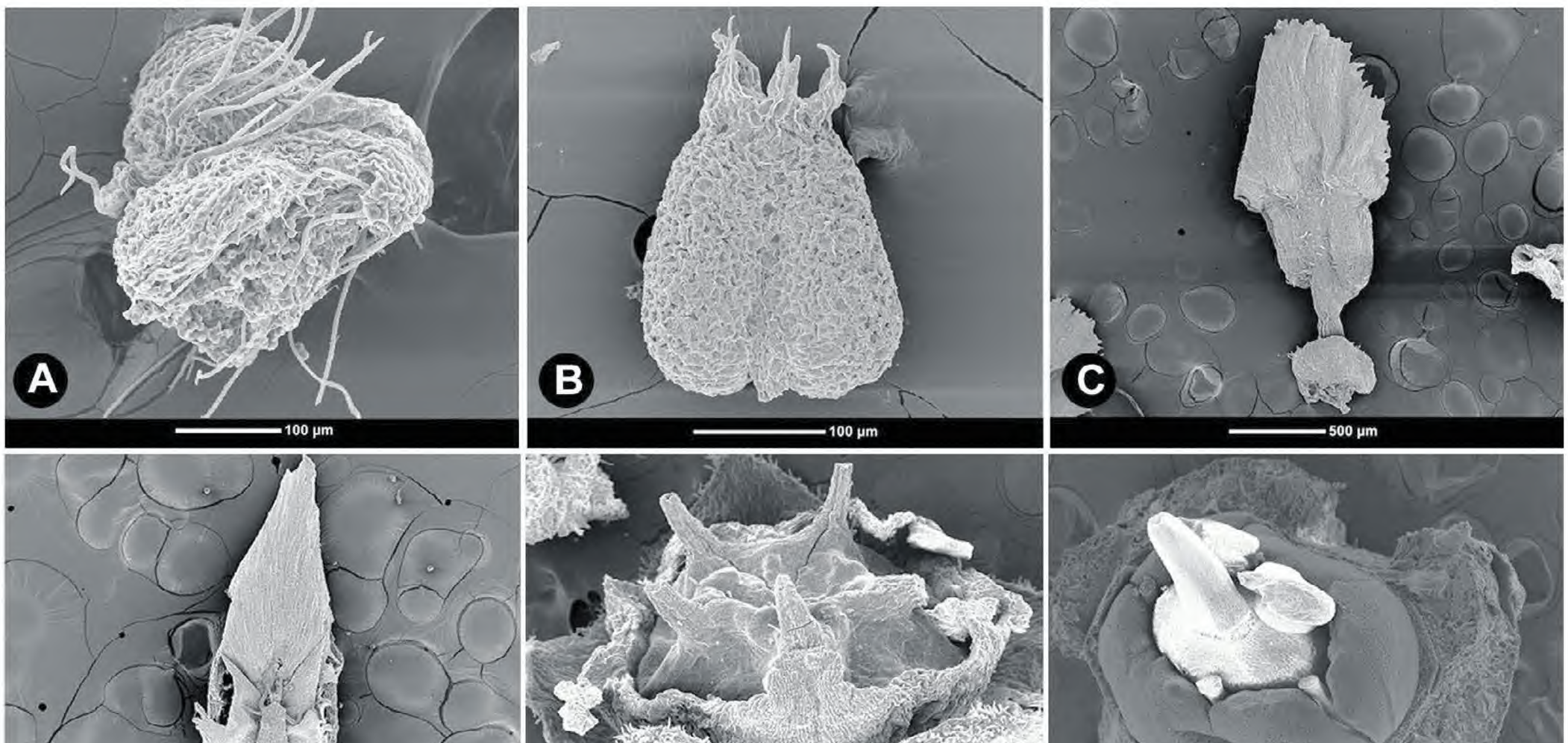


Figure 4. SEMs of androecia and gynoecia among representative *Rinorea* Aubl. and *Fusispermum* Cuatrec. (Violaceae) and *Goupia* Aubl. (Goupiaceae, outgroup). —A. Androecium, *G. glabra* Aubl. (*B. E. Hammel 21278*). —B. Androecium, *F. laxiflorum* Hekking (*G. S. Hartshorn 2929*). —C. Androecium, *R. crenata* S. F. Blake (*M. H. Grayum 10069*). —D. Androecium, *R. lindeniana* (Tul.) Kuntze (*S. E. Hoyos-Gómez 999*). —E. Gynoecium, *G. glabra* (*B. E. Hammel 21278*). —F. Gynoecium, *F. laxiflorum* (*Hartshorn 2929*). —G. Gynoecium, *R. crenata* (*M. H. Grayum 10069*). —H. Gynoecium, *R. viridifolia* Rusby (*S. E. Hoyos-Gómez 1003*). —I. Gynoecium, *R. lindeniana* (*S. E. Hoyos-Gómez 1127*). Scale bars in A, B = 100 µm; C, F, I = 500 µm; D, E, G = 200 µm; H = 1000 µm. All collections are at MO.

ANDROECIA

In all 11 species studied, stamen filaments were glabrous. In *Goupia glabra* these filaments were ca. 200 µm long, and the anther thecae were ca. 260 µm long; in *Fusispermum laxiflorum* they were ca. 500 µm and 240 µm long, respectively. In the seven *Rinorea* species examined the filaments ranged from ca. 300 µm to 950 µm long, and the anther thecae were comparatively longer than in *G. glabra* and *F. laxiflorum*, ranging from ca. 530 µm to 1100 µm long. *Goupia glabra* was the only species to have trapezoid anther shapes (Fig. 4A); the remaining 10 species exemplars had ellipsoid to ovoid anthers. In all species dehiscence was introrse via longitudinal slits in each theca.

In *Goupia glabra*, the androecial connective formed a thick, blunt prolongation beyond the thecae ca. 90 µm long, with cylindrical hairs ca. 230 µm long that were scattered on both the connective and thecae (Fig. 4A). In *Fusispermum laxiflorum* (Fig. 4B), *F. minutiflorum*, and *F. rubrolignosum*, the prolongation of the connective was less massive, ca. 80 µm long, with an irregular fringe apically, while in *Rinorea apiculata* and *R. crenata* (Fig. 4C) the connective formed a broad apical prolongation that exceeded the thecae in length, ca. 930–1100 µm long, with a fimbriate margin. In the remaining five *Rinorea* species sampled, the thecae were not evident from the dorsal side; the connective formed a slightly fringed acute to triangular projection ca. 1300–2000 µm long. Only in *R. apiculata* and *R. crenata* (Fig. 4C) were there flattened hairs on both the adaxial and abaxial surfaces of the anther thecae; in *R. squamata*

there were scattered cylindrical hairs adaxially on the thecae. The remaining Violaceae exemplars had glabrous anthers.

In *Goupia glabra* and *Fusispermum laxiflorum*, *F. minutiflorum*, and *F. rubrolignosum*, an apparently warty disc-like structure with epidermal stomata surrounded the stamens but was quite separate from the anthers. In *G. glabra* this lobed disc completely surrounded the flower (Fig. 4E), with the lobes alternating with the staminal radii. In *F. laxiflorum* (Fig. 4F), *F. minutiflorum*, and *F. rubrolignosum*, the massive disc consists of five lobes alternating with the stamens.

By contrast, the seven examined species of *Rinorea* lacked a disc but did have an abaxial gland-like structure at the base of the filaments; this has hairs in *R. lindeniana* (Fig. 4D), *R. viridifolia*, and *R. paniculata*. In both *R. apiculata* and *R. crenata* (Fig. 4C), this gland was massive, ca. 190 μm long, hemispherical, and joined at the very base with adjacent glands. In the other five species of *Rinorea*, glands were distinct from one another and borne on the filament. In the 11 species studied, there were stomata on the disc and glands consistent with their being nectariferous.

GYNOECIA

Goupia glabra has a glabrous gynoecium of five carpels with five styles borne toward the margin of the carpels (Fig. 4E). Each style, only ca. 200 μm long, has an adaxial furrow and tapers somewhat toward a perhaps hollow but otherwise undistinguished apex. Placentation is axile with ca. five to six ovules per carpel.

All 10 species exemplars of the Violaceae had three fused carpels with parietal placentation. There were many ovules per carpel in *Fusispermum laxiflorum*, *F. minutiflorum*, and *F. rubrolignosum*, but only one ovule per carpel in the seven species of *Rinorea* examined. In *F. laxiflorum* (Fig. 4F), *F. minutiflorum*, *F. rubrolignosum*, *R. apiculata*, and *R. crenata*, the ovary was glabrous, but in other species such as *R. viridifolia* (Fig. 4H), the ovary was pubescent. Styles of all exemplars were continuous with the ovary. In *F. laxiflorum* (Fig. 4F), *F. minutiflorum*, and *F. rubrolignosum* the straight style was narrowed distally to the apex, which had an inconspicuous depression, while in *R. apiculata* and *R. crenata* (Fig. 4G) the style was conspicuously flared at the apex. *Rinorea viridifolia* and *R. squamata* had straight styles and stigmas rather like those of *F. laxiflorum* (Fig. 4F). *Rinorea lindeniana* and *R. paniculata* had sigmoid styles, subapically swollen in *R. lindeniana*, but in both species the stigmatic apex resembled that of the other species of *Rinorea* examined.

SEEDS

The outgroup representative *Goupia glabra* had dark brown ovate seeds ca. 2–3 mm long (Fig. 5A), with the anticlinal walls of the exotesta forming a reticulum (Fig. 5B, C). In transverse section the exotesta was two, perhaps three, cell layers thick and ca. 60–80 μm across; the outer periclinal walls of the exotesta were slightly thickened. There was also a single layer of exotegmic sclereids, these cells being ca. 60 × 100 μm and massively thickened on the anticlinal and inner periclinal walls and also pitted (Fig. 5B, C). *Goupia glabra* lacked crystals in the seed coat. Its straight embryo occupied half the length of the seed and was surrounded by copious oily endosperm.

In contrast, *Fusispermum minutiflorum* (Fig. 5D) and *F. rubrolignosum* had pale brown elongated seeds, ca. 2–3 mm long. The seed had what appears to be a wing down one side where the seed coat was much thicker and was ca. 11 to 16 cells across. There was also a single layer of exotegmic sclereids, the cells being ca. 100 × 10 × 15 μm, and the cell walls being massively lignified all around and pitted (Fig. 5E). There were druses in the testa, as were seen in all seven *Rinorea* species studied. The straight embryo occupied 3/5 the length of the seed, and an oily endosperm surrounded the embryo (Fig. 5D).

Rinorea apiculata and *R. crenata* (Fig. 5G) have light brown ovoid seeds, ca. 4–5 mm long. In transverse section, the testa consisted of unlignified, more or less isodiametric cells three to four layers thick. There was a single layer of exotegmic cells ca. 150 × 10 × 70 μm (Fig. 5F, *R. apiculata*). These cells had more or less pitted walls that were all equally thickened. Only in *R. apiculata* and *R. crenata* among the seven species studied here were there rhombic crystals in the testa. The straight embryo occupied four fifths of the length of the seed and was surrounded by copious oily endosperm surrounding the embryo.

Rinorea squamata, *R. viridifolia* (Fig. 5H), and *R. dasyadena* had brown globose seeds ca. 6–9 mm across. The testa consisted of eight to 10 layers of unlignified flattened cells, with individual cells isodiametric and ca. 20 μm across (Fig. 5H, *R. viridifolia*). There is a middle layer of lignified cells about four to six cells across, the cells being ca. 190 × 15 × 30 μm (Fig. 5H). The cell walls were equally thickened and more or less consistently pitted. The embryo occupied four fifths of the length of the seed,

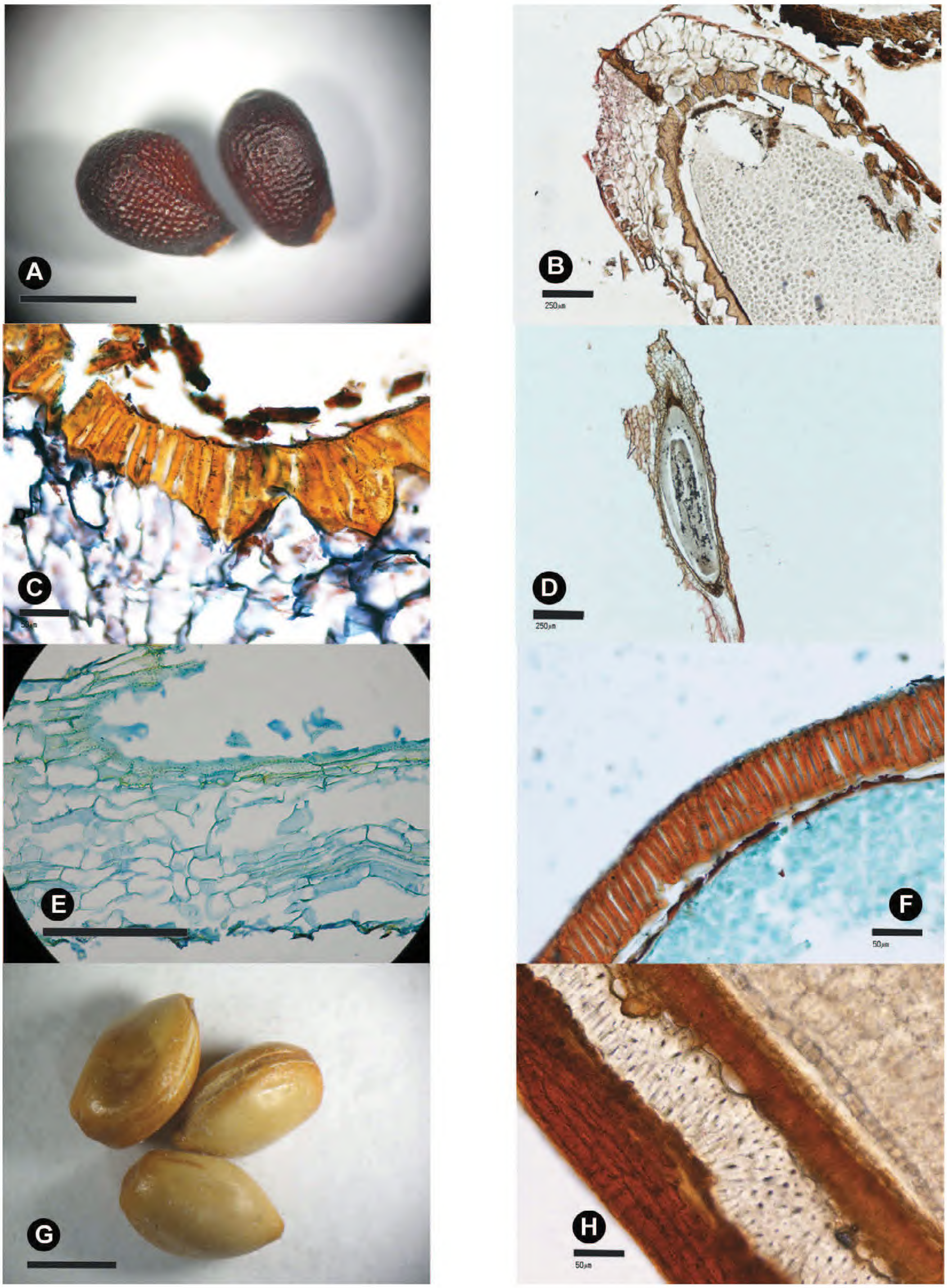


Figure 5. Light microscopic images of seeds and transverse sections among *Fusispermum* Cuatrec. and *Rinorea* Aubl. (Violaceae) and *Goupia glabra* Aubl. (Goupiaceae). —A. Two intact seeds, *G. glabra*. —B. Transverse seed section, *G. glabra*. —C. Transverse seed section, *G. glabra*. (A–C taken from *T. W. Henkel 3245*.) —D, E. Transverse seed sections, *F. minutiflorum* Cuatrec. (*J. Espina 1330*). —F. Transverse seed section, *R. apiculata* Hekking (*C. E. Cerón 5478*). —G. Three intact seeds, *R. crenata* S. F. Blake (*B. E. Hammel 18113*). —H. Transverse seed section, *R. viridifolia* Rusby (*S. E. Hoyos-Gómez 1004*). Scale bars in A = 3000 µm; B–E = 250 µm; F, H = 50 µm; G = 2000 µm. All collections are at MO.

and the cotyledons had several sinuous foldings; copious oily endosperm surrounded the embryo.

Discussion

The results of this study suggest a number of morphological and anatomical characters that are useful for understanding the Neotropical relationships among the Goupiaceae (i.e., *Goupia glabra*) and the two basalmost genera among the Violaceae (i.e., *Fusispermum laxiflorum*, *F. minutiflorum*, and *F. rubrolignosum*; *Rinorea apiculata*, *R. crenata*, *R. lindeniana*, *R. squamata*, *R. viridifolia*, *R. dasyadena*, and *R. paniculata*). Several significant character states (16) are mapped on a summary tree diagram for phylogenetic relationships among these Violaceae and Goupiaceae (Fig. 1). Observations are confirmed here, often for the first time, and are integrated with the sparse literature available (cf. Metcalfe & Chalk, 1972, anatomy; Corner, 1976, seeds).

Overall, these results show the value of anatomical studies when related to a phylogeny. Nodal anatomy is a good example. *Goupia glabra* was considered to belong to Celastraceae by some authors based on gross morphology (Cronquist, 1981). However, the nodes of Celastraceae are overwhelmingly unilacunar (Sinnott, 1914; Cronquist, 1981). The three-trace, three-gap nodes of *G. glabra* are more consistent with its new position, more so since other Celastraceae with other than unilacunar nodes (e.g., *Perrottetia* Kunth, *Bhesa* Buch.-Ham. ex Arn.) have also been removed from Celastraceae to clades where these nodes are common (Stevens, 2012). Furthermore, the pentalacunar nodes of *Fusispermum laxiflorum* are uncommon in Malpighiales and are a potential apomorphy for the genus, and it can be noted here that nodal anatomy is associated with a distinctive petiole anatomy.

The fluted stem pith of *Goupia glabra* is an apparently trivial difference, which is nevertheless consistent within the Goupiaceae and contrasts with the cylindrical pith seen within those Violaceae analyzed (see also Metcalfe & Chalk, 1972). Although little more is known about pith shape in the Achariaceae, it has been characterized as circular (Metcalfe & Chalk, 1972), so that the fluted stem pith of *Goupia* may be regarded as an apomorphy, and stem pith shape is mapped on Figure 1 (character states 1, 2). Metcalfe and Chalk (1972: 103) say of Violaceae that "the pith is solid in woody species, but frequently becomes hollow in herbs"; however, there is no mention of heterogeneous pith. The heterogeneous pith of *Fusispermum* is potentially an apomorphy for the genus.

As Howard (1974, 1979a, 1979b) emphasizes, the vascular anatomies of stem, petiole, and blade midrib cannot be considered in isolation. Bearing this in mind, I nevertheless particularize some observed variations to facilitate the discussion. Sinnott (1914) previously reported three-trace, three-gap nodal anatomy for the Violaceae, Passifloraceae, and Flacourtiaceae s.l., in general for plants with stipules. Such trilacunar stem nodes are found throughout the Malpighiales, with the exception of the Bonnetiaceae–Calophyllaceae clade (cf. Stevens, 2012). The eight species studied here for *Rinorea* and *Goupia* shared these trilacunar nodes, with the exception of *Fusispermum*, which had pentalacunar stem nodes. In the context of Malpighiales, these pentalacunar nodes are likely to be an apomorphy and are mapped as such (Fig. 1).

The most conspicuous gross variation for leaf midrib anatomy in the species studied here was observed for the number of adaxial to lateral bundles and the tissue configuration in the central vascular bundle of the midrib. Although *Goupia glabra* and *Fusispermum laxiflorum* had medullary plates of vascular tissue associated with the central bundle, the midrib vasculature was very different in morphology. Metcalfe and Chalk (1972) mention that in *G. glabra*, xylem was adaxially presented and phloem abaxial (1972). However, in *F. laxiflorum*, xylem entirely surrounded phloem in the latter, which is rather unusual and probably an apomorphy (Fig. 2H). *Rinorea apiculata* has an open central bundle, while the midrib vasculature in the other *Rinorea* species examined is annular.

Although this anatomical variation was not optimized on the summary tree, the structure of the petiole bundle (and associated nodal anatomy) throughout the family will require further examination. For the herbaceous *Viola*, Metcalfe and Chalk (1972) report an arcuate central vascular strand in the petiole accompanied by one or two rib strands on each side. There are possible correlations with leaf insertion. Thus, *Fusispermum laxiflorum*, *F. minutiflorum*, *F. rubrolignosum*, *Rinorea apiculata*, *R. crenata*, and *R. paniculata*, all with distichous phyllotaxy, have two pairs of rib bundles. It will be interesting to see if other species in *Rinorea* s. str. that have been reported to have such ranked or alternate leaves have similar vasculature. It is confirmed here that *R. viridifolia*, *R. dasyadena*, and *R. squamata*, which have opposite leaves and symmetric lamina bases, have only a single rib bundle on either side of the petiole. *Rinorea lindeniana*, with two rib bundles on one side and one on the other (Fig. 3E), presents an interesting

anomaly. It has distichous leaves with very strongly asymmetric lamina bases, which may account for the odd number of vascular bundles.

All 10 species of the Violaceae (*Rinorea*, *Fusispermum*) studied here had calcium oxalate crystals (as rhombic crystals and druses) in the collenchyma. Only *Goupia glabra* lacked such rhombic crystals of calcium oxalate in the collenchyma tissue.

The thickness of the leaf lamina found here for the 10 Violaceae sampled may correlate with environmental conditions (Dickison & Weitzman, 1996; Alvarenga & Lombardi, 2010). *Fusispermum laxiflorum*, with the thickest blades at 270 μm, can reach the forest canopy, being a tree up to 20 m or more, while those *Rinorea* species studied are understory species from 2 to 10 m tall. In the sampled species, the leaves of *Rinorea* ranged from 150 to 180 μm, and these trees never reached the canopy. Both *Goupia glabra* and *F. laxiflorum* had similar leaf blades with well-developed palisade tissue as sampled, and both were trees that reach the canopy. The *Rinorea* species studied have poorly organized palisade tissue, and all were collected from smaller plants. It should be noted that *R. apiculata* can grow to heights of 30 m (Hekking, 1988). Palisade tissue tends to be better developed under well-insulated conditions (e.g., Dickison & Weitzman, 1996; Alvarenga & Lombardi, 2010). Some species of *Viola* have been noted to have no clearly differentiated palisade tissue (Metcalfe & Chalk, 1972), but the genus is herbaceous and may occur in somewhat shaded conditions.

The structure of the midrib largely mirrored that of the petiole for the 11 species sampled. In *Rinorea apiculata*, with its incurved-arcuate primary petiole bundle, the midrib was fully bilayered, as in the other species of *Rinorea* s. str. examined. In *Goupia glabra* the midrib was also bilayered, evidence of the petiolar medullary plate being lost except for the presence of a few medullary fibers. In *Fusispermum laxiflorum*, *F. minutiflorum*, and *F. rubrolignosum*, the vascular tissue in the midrib consisted of two arcuate bands of fibers surrounding the vascular tissue, with two additional bands of vascular tissue in the midrib that were continuous with the elliptical central cylinder in the petiole.

The stamens of many Violaceae are notable for the abaxial nectar glands on some or all filaments and the complex, flattened connective (Arnal, 1945). Species of *Rinorea* s. str. have glands adnate to the androecial filament that can be either fused or free, and the variation is taxonomically significant.

Assuming that the Goupiaceae are sister to Violaceae, there appears to be an increasing elaboration of the androecial connective and a shift in the nectary from a structure more or less separate from other parts of the flower to its adnation to the filament in basal Violaceae, as is seen in the *Rinorea* exemplars, even though polarization of the anatomical variation was difficult to distinguish. The rather massive disc surrounding the staminal whorl in *Goupia glabra* and *Fusispermum laxiflorum* is similar to the nectaries of other members of the parietal placentation group in the Passifloraceae, Violaceae, and Salicaceae (Stevens, 2012), with the nectary lobes alternating with the filaments. For the seven species of *Rinorea* examined, the nectary was more specifically associated with the abaxial part of the filament. The enlarged, hemispherical disc of the *R. apiculata* group (*R. apiculata*, *R. crenata*, and *R. oraria*) is morphologically similar to the thickened disc of *Fusispermum* and *Goupia*, while in the other species of *Rinorea* s. str. the nectariferous tissue is more closely associated with the filament, which is the more common condition seen elsewhere in the family.

These characters—the nectary lobes alternating with or opposite to filaments, the nectary disclike, and the gland adnate to the filament—are included on the tree (see Fig. 1). However, more detailed comparative studies on the stamens/nectaries in Violaceae are likely to disclose more phylogenetically interesting information.

The anther connective is stout and rounded in *Goupia glabra* but flattened and relatively thin in all other species sampled. *Fusispermum laxiflorum, F. minutiflorum*, and *F. rubrolignosum* have a very short, erose connective ca. 80 μm long, while the longest connective occurs in *Rinorea viridifolia* (ca. 2 mm long). In the *R. apiculata* group (*R. apiculata*, *R. crenata*, and *R. oraria*), the anther thecae are visible in adaxial view, while in the other species they are concealed. The connective scales are known to vary in size and shape among other Violaceae (Engler, 1925; Arnal, 1945).

Variation in the gynoecia of the 11 species sampled is of considerable interest. The axile placentation and basal ovules of the outgroup *Goupia glabra* (see, e.g., Miers, 1862) are unique among the parietal placentation group in the Malpighiales and may be hypothesized as apomorphies, as are the 5-carpellate gynoecium and marginal styles (uncommon in Malpighiales). However, details of the stigmatic area of both *Goupia* and those Violaceae sampled may well be similar: the stigmatic area is not expanded, and there is a central depression or pore noted in all (Fig. 4E, F, H). More noteworthy in the *Rinorea apiculata* and *R. crenata* (Fig. 4G), the apex of the style flares outward a likely apomorphy.

Most Violaceae studied had straight styles (Fig. 4H), but in *Rinorea lindeniana* the style is curved at the base (Fig. 4I), and in *R. paniculata* the style is sigmoid at the base. In genera such as *Hybanthus*, *Amphirrhox* Spreng., *Paypayrola* Aubl., and *Gloeospermum* Triana & Planch., the styles are straight, consistent with the family (Steyermark et al., 2005). In *Rinoreocarpus ulei* (Melch.) Ducke and other species of *Rinorea* and *Viola*, the style has been noted as slightly curved (Engler, 1925; Arnal, 1945; Hekking, 1988). The taxonomic significance of the style curvature needs further investigation within the family.

Of the species studied, only *Goupia* and *Fusispermum* have more than a single ovule per carpel (Hekking, 1984), which is probably the plesiomorphic condition for Violaceae as a whole. Although the seven species of *Rinorea* examined consistently had only one ovule per carpel, there is considerable variation in ovule number in other Violaceae, and the phylogenetic utility of the character is not clear.

It has been suggested that the flowers of *Rinorea* (see Arnal, 1945) and *Fusispermum* (Hekking, 1988) species are actinomorphic. Recent studies of floral development in *Rinorea* demonstrate that the polysymmetry of this actinomorphy is achieved late in the ontogeny. Almost actinomorphic to very weakly zygomorphic flowers are noted in *Gloeospermum* (Steyermark et al., 2005), and flowers that are actinomorphic in early stages of development in *Leonia* Ruiz. & Pav. become zygomorphic later (Feng, 2005). Feng and Ballard (2005) suggest that the family had a most recent common ancestor with zygomorphic monosymmetric, rather than actinomorphic, flowers. However, early developing flower buds of *F. laxiflorum* and two species of *Rinorea* (i.e., *R. lindeniana* and *R. dasyadena*) have been seen to be zygomorphic with a more or less oblique orientation (Hoyos-Gómez, pers. obs.), which might suggest that zygomorphic flowers may indeed be a synapomorphy for the Violaceae.

There is interesting variation in the seed coat among the Violaceae, and studies by Corner (1976), Plisko (1992), and Melikian and Sarinov (2000) have placed this variation in phylogenetic context. When studying seed coat anatomy, ideally one should look at developmental stages so that one can follow the development of particular portions of the integuments as incorporated into the seed coat (e.g., Corner, 1976). Such developmental series could not be observed here, so the attribution of cells to the tegmen is inferred on the number of layers to the testa, the presence of a cuticle, and so forth.

The filiform seeds of *Fusispermum minutiflorum* have longitudinal ridges or wings 11 to 16 cells across, with the embryo occupying about half the length of the seed. This differed from the ovoid to ellipsoid seeds of the other taxa examined here and also differed from seeds of the Achariaceae, Passifloraceae, and the like (Malpighiales). The winged, filiform seed shape noted for species of *Fusispermum* can be considered an apomorphy and is linked to differences in seed coat anatomy.

The exotegmen of the seed wall consisted of a more or less well-developed layer of lignified cells, all cell walls of which were more or less evenly thickened in the *Fusispermum* and *Rinorea* species sampled (but not in *Goupia glabra*), and there were fine plasmodesmata evident across the cell walls (Fig. 5E, F). In *G. glabra* the testa was at least two to three cell layers across, and there was a single layer of massively cubical cells, presumably exotegmic (Fig. 5C). In the *R. apiculata* group (with *R. crenata*) the testa was three to four cell layers across, and the sclerified layer consisted of a single layer of obliquely overlapping cells (Fig. 5F). In the other five species of *Rinorea* s. str. examined, the seed coat was eight to 10 cell layers across, of which four to six layers were lignified, the cells being elongated and flat lying (Fig. 5H).

A single layer of sclerified cells in the exotegmen was found in all Violaceae studied by Corner (1976), Plisko (1992), and Melikian and Sarinov (2000), including the berry-fruited *Leonia*. But for species like *Rinorea anguifera* Kuntze (Plisko, 1992), four to five layers of sclerified cells were observed. In this study, *R. dasyadena*, *R. lindeniana*, and *R. squamata* have similar, multilayered sclerified tegmic tissue. Note that Melikian and Sarinov (2000) show the seed coat of *Goupia glabra* as having mesotestal sclereids. However, although the material examined here was limited, there was clearly more than one layer of cells above the sclerified layer, suggesting that the sclerified layer is exotegmic. This layer was also definitely longitudinally ridged, another feature not noted by Melikian and Sarinov (2000), who placed the Goupiaceae near Celastraceae, which would commonly have a lignified mesotesta.

Some kind of exotegmen made up of more or less fibrous cells is common in the Malpighiales (e.g., Corner, 1976; Stevens, 2012). Details of cell elongation and the degree, and plane, of the cellular flattening have varied and need to be integrated with phylogeny across the clade. However, despite our still limited knowledge of seed coats in the Violaceae, the four or five layers of fibrous cells found in the species

of *Rinorea* s. str. studied, as a provisional finding, can be considered unique.

The embryo in *Goupia glabra*, *Fusispermum minutiflorum* (Fig. 5D), and the *Rinorea apiculata* clade was straight, with flat cotyledons, a condition considered to be normal for the family (e.g., Plisko, 1992). However, in the other examined species of *Rinorea* s. str., the cotyledons exhibited complex folds. Vogel (1980) noted that *Rinorea* species had seedling type I (*Macaranga* Thouars–type), where cotyledons in most cases were more or less folded within the seed. Further investigation of embryo morphology would repay the effort with characters of phylogenetic significance.

Examination of the androecium and nectary in those Violaceae and Goupiaceae examined relates the distinctive androecium so common in Violaceae with more conventional structures found in other members of the parietal placentation group within the Malpighiales. Although many features of the gynoecium of *Goupia* are quite different from those of the parietal classification group, the two families had not been previously associated, not until support for the sister relationship of the Goupiaceae to the Violaceae was corroborated by molecular evidence. Otherwise, in the morphology of the stigma and androecial features, as well as in basic anatomy, the Violaceae and Goupiaceae agree quite well. Without such detailed anatomical and morphological knowledge we cannot hope to understand evolution and diversification, not only of Violaceae but of Malpighiales as a whole, such an important component of the small trees that are so abundant in tropical rainforests (Davis et al., 2005).

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BOTANY OF THE 1841 FRÉMONT–GEYER EXPEDITION TO THE DES MOINES RIVER[1]

Thomas G. Lammers[2]

ABSTRACT

In June and July 1841, Lt. John Charles Frémont of the United States Army Corps of Topographical Engineers led a survey of the lower Des Moines River in the territory of Iowa and the state of Missouri, accompanied by botanist Karl Andreas Geyer. The resulting publication was the earliest to report on the flora of Iowa in any sort of scientific fashion. Frémont described the major plant communities encountered, in the process mentioning 30 species: 24 trees and shrubs of the forest and six herbs of the prairie. Seven herbaceous species not mentioned in the report are documented by specimens in the herbarium of Missouri Botanical Garden.

Key words: Botanical exploration, botanical history, Des Moines River, George Engelmann, Iowa, John Charles Frémont, Joseph Nicolas Nicollet, Karl Andreas Geyer, Missouri.

John Charles Frémont (1813–1890) looms large in American history (Warren, 1859; Wilson & Fiske, 1888; Dellenbaugh, 1914; Nevins, 1928; Phillips, 1935; Egan, 1977; Chaffin, 2002): exploring and mapping the Rocky Mountains, the Oregon Country, the Great Basin, and the Sierra Nevada; helping to wrest California from Mexico and shepherding it to statehood; convicted on charges of mutiny, disobedience of a superior officer, and military misconduct; standing as the Republican Party's first candidate for President; emancipating Missouri's slaves while serving as commanding general of the Army's Department of the West. Among botanists, he is known as the eponym or author of numerous names bestowed on plants discovered during his explorations: *Fremontodendron* Coville, *Oxystylis* Torr. & Frém., *Amsonia tomentosa* Torr. & Frém., *Gentiana fremontii* Torr., *Pinus monophylla* Torr. & Frém., *Populus fremontii* S. Watson, *Prunus fremontii* S. Watson, and *Senecio fremontii* Torr. & A. Gray, to name but a few.

Posterity has decreed that Frémont's 1842 exploration of the Rocky Mountains was his first expedition (Ewan, 1950; Jackson & Spence, 1970; Schubert, 1980; Welsh, 1998), and the explorer himself considered it such (Frémont 1887: 73). It and his four subsequent expeditions were written up in great detail (Frémont, 1843, 1845a, 1845b, 1846, 1848, 1853) and publicized widely (Benton, 1850; Torrey, 1853; Bigelow, 1856; Smucker, 1856; Upham, 1856; Warren, 1859). However, the summer before this supposedly first expedition, he led an expedition to the lower Des Moines River in the territory of Iowa and state of Missouri. This earlier expedition generated but a single brief report buried among Congressional debate of the Iowa–Missouri border dispute (Anonymous, 1842), and Frémont's biographers seem to mention it only because of the role it played in his courtship and marriage (see below). Iowa's historians likewise have accorded it scant attention compared to similar military reconnaissances of the state (Lea, 1836, 1890; Allen, 1846; van der Zee, 1913; Pelzer, 1917; Hurt, 1998).

In all his expeditions, Frémont paid special attention to the plant life of the areas surveyed, and this was just as true for the Des Moines River expedition. As a result, his published report (Frémont, 1842) was the first to describe the flora of Iowa in any sort of scientific fashion. However, like Rauch's (1851) first checklist of the state flora (Lammers, 2013), Frémont's report has not been mentioned in the Iowa floristic literature (e.g., Bessey, 1871; Fitzpatrick, 1899; Gilly, 1947; Thorne, 1954; Eilers, 1975; Eilers & Roosa, 1994; Lewis, 1998; but cf. Yatskievych, 1999). It is all but unknown to the state's botanists, although Iowa's historians learned of it almost a century ago (van der Zee, 1916; Shambaugh, 1918). The objective of this

[1] My sincerest thanks to John Pearson of the Conservation and Recreation Division, Iowa Department of Natural Resources, for kindly calling my attention to Shambaugh's (1918) reprint of the Frémont (1842) and Guion (1842) reports; the present paper would never have been written if not for this simple act of courtesy. Thanks also to the curatorial staff in the herbarium (MO) at Missouri Botanical Garden for their devoted assistance when I visited to search for Geyer specimens from Iowa; to my wonderful wife Diane for her invaluable assistance during this search; and to Charles E. Umbanhowar, Jr., of St. Olaf College for helpful discussions on Geyer's collecting efforts in general.

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paper is to collate all available information on the expedition to the Des Moines River, as an avowed supplement to Welsh's (1998) comprehensive account of the botany of Frémont's five subsequent expeditions, in order to assure for it the place it deserves in Iowa's botanical history and in the Great Pathfinder's legacy.

Prior Surveys by Frémont

To give context to Frémont's survey of the Des Moines River, a brief overview of his prior work in this line is provided. This has been synthesized from his autobiography (Frémont, 1887) and various biographical sources (Benton, 1850; Bigelow, 1856; Smucker, 1856; Upham, 1856; Wilson & Fiske, 1888; Dellenbaugh, 1914; Nevins, 1928; Egan, 1977; Welsh, 1998; Chaffin, 2002). These primary sources, which should be consulted for additional information, are not cited further in this section.

In 1833, former Congressman Joel Roberts Poinsett (1779–1851) of Charleston, South Carolina, assisted a young man of his acquaintance, John Charles Frémont, in securing an assignment as an instructor of mathematics aboard the 200-ton sloop-of-war U.S.S. *Natchez*. When that assignment ended, Poinsett helped Frémont obtain a position as an assistant to Capt. William G. Williams (1801–1846) of the United States Army Corps of Engineers. Williams was then engaged in surveying a route through mountain passes on the North Carolina–Tennessee border for the proposed Louisville, Cincinnati & Charleston Railroad. When work on the line was suspended, Williams hired Frémont to assist him on his next assignment, a military reconnaissance of Cherokee lands in Georgia and adjacent states. Frémont later wrote that it was during this period, while working with Williams, that he found the path in life that he was "destined to walk" (Frémont, 1887: 24).

In 1837, Frémont's benefactor Poinsett was appointed Secretary of War in the Cabinet of President Martin Van Buren (1782–1862). With a letter of recommendation from Capt. Williams, Frémont wrote to Poinsett, seeking a commission in the Army as a topographical engineer. When the Cherokee project concluded, Frémont was ordered to Washington. The Department of War had contracted with French cartographer Jean (Joseph) Nicolas Nicollet (1786–1843) to prepare a detailed map of the upper Mississippi River valley, and Frémont was to be his assistant.

However, when the young surveyor left Washington on that assignment in April 1838, it was as a civilian employee, under the provisions of the act of 30 April 1824 (*4 Stat., 46*). His commission as a second lieutenant in the United States Army did not come about until passage of the act of 5 July 1838 (*5 Stat., 162*), which established the Corps of Topographical Engineers as an entity distinct from the Corps of Engineers and authorized staffing its ranks with its former contractors (Beers, 1942; Schubert, 1980). Frémont spent the 1838 and 1839 field seasons assisting Nicollet in what is today Wisconsin, Minnesota, and the eastern Dakotas (Bray, 1970, 1980; Bray & Bray, 1976).

In addition to Nicollet and Frémont, the survey party included Karl (Charles) Andreas Geyer (1809–1853), a German botanist who had emigrated from Saxony to New York in 1834 (Torrey, 1843, 1845; Geyer, 1845; Reichenbach, 1855; Spaulding, 1909; Nute, 1945; McKelvey, 1955; Heinz, 1989). In 1835, Geyer had botanized on the Big Nemaha River and the North Fork of the Platte River in what is today Nebraska. Returning to St. Louis via steamboat, he met Nicollet. Nicollet was so impressed by the young German that when he was assigned to map the upper Mississippi watershed, he offered to pay Geyer out of his own pocket to document the flora of the region. Geyer declined the offer in 1836–1837, but did take part in the 1838–1839 expeditions. That first autumn, during a visit to Spirit Lake in what is now Dickinson Co., Iowa, Geyer collected some of the earliest herbarium specimens known from the state (Shimek, 1915; Eilers, 1975; Bray & Bray, 1976; Lammers, 1985, 2013); these were identified and described by Torrey (1843, 1845).

Nicollet and Frémont returned to Washington in December 1839 to begin work on the map (Nicollet, 1843a) and the accompanying report (Nicollet, 1843b, 1845) at the Bureau of the Corps of Topographical Engineers. Proponents of westward expansion were much interested in the map, and frequently called at the Bureau to view its progress. Among the many men of influence whom Frémont met in this way was Sen. Thomas Hart Benton (1782–1858) of Missouri. Like Poinsett before him, the senator would become a major figure in the development of the young topographer's career. And when Frémont later met the senator's 15-year-old daughter, Jessie Ann Benton (1824–1902), it was love at first sight. Her parents, while holding the young lieutenant in high personal regard, were opposed to his suit, in part because of Jessie's youth and in part because of the vagaries of a military career; they insisted upon a one-year moratorium of the courtship.

On 4 June 1841, Frémont received orders from his superior at the Bureau, Col. John James Abert (1788–

1863), to "repair without delay to the mouth of the Racoon [sic] fork of the Des Moines, in order to determine that position, and the Topography of the adjacent country," and to make a survey of the Des Moines from there to its mouth (Jackson & Spence, 1970: 96). To Frémont, this assignment represented advancement, as it was his first opportunity to direct a survey himself instead of merely assisting another topographer (Frémont, 1887).

Rationale for the Des Moines River Survey

More has been written on the rationale behind the Des Moines River survey than on the events of the survey itself. Many of Frémont's biographers indicate that issuance of the order was occasioned by his courtship of Miss Benton, that it was simply a ploy to take an unsuitable suitor out of the picture. Frémont (1887: 68) himself believed this to be the case, writing, "Whether or not this detachment of myself from Washington originated with Mr. Nicollet I do not know, but I was loath to go ... the survey was a health-giving excursion, but it did not cure the special complaint [i.e., his fondness for Miss Benton] for which I had been sent there."

Some suggest that Sen. Benton himself used his political influence to arrange Frémont's dispatch (Wilson & Fiske, 1888; McKelvey, 1955). Others point to his wife, Elizabeth McDowell Benton (1794–1854), and her close friendship with Mary Izard Pringle Poinsett (1780–1857), the wife of Joel Roberts Poinsett (see above), as the origin of the ruse (Nevins, 1928; Phillips, 1935). However, Poinsett's tenure as Secretary of War ended on 5 March 1841 with the inauguration of President William Henry Harrison (1773–1841), and it was not until this time that the Bentons truly became alarmed at the depth of their daughter's feelings for the young lieutenant (Dellenbaugh, 1914; Egan, 1977). By the time Frémont was ordered to Iowa Territory three months later, Poinsett had been replaced as Secretary of War by John Bell (1796–1869). However much Poinsett may have sympathized with the Bentons, it would seem that he was not in a position to effect such an order.

On the other hand, Jackson and Spence (1970) were skeptical of the idea that Frémont's affection for Jessie was the raison d'être for the Des Moines River survey. Instead, they pointed to the ongoing border dispute between the territory of Iowa and the state of Missouri, which hinged on understanding the hydrologic feature long known as the Rapids of the Des Moines (Anonymous, 1842; Edwards, 1843; Avery, 1894; Larzelere, 1916): was it situated in the Des Moines River itself, or in the Mississippi River near the mouth of the Des Moines? Jackson and Spence (1970) suggest that Benton counted on Frémont's survey to support the former position and thus ensure a placement of the boundary more favorable to Missouri.

However, nothing in Frémont's orders or in his report dated 19 April 1842 speaks to the border dispute. His comments on rapids on the Des Moines River appear only in an addendum written on 10 December 1842, a year and a half after the survey, in response to a direct query from Rep. John Cummins Edwards (1804–1888) of Missouri. Here he described his examination of them as "merely incidental" (Frémont, 1842: 19). Nevertheless, Edwards (1843) utilized carefully edited snippets of Frémont's report in a speech favoring a more northerly boundary. Congress was unable to resolve the issue, and it was not until 1849 that the current border was established by the United States Supreme Court (*State of Missouri v. State of Iowa, 48 U.S. 660*).

One aspect of the survey I have not seen discussed: whether there actually was a need for the data gathered by Frémont. If indeed there was, then there was nothing about the order that was "mysterious" (Bigelow, 1856: 34). Nicollet himself was at that time too ill to travel (Nicollet, 1843b, 1845; Bray, 1970, 1980; Bray & Bray, 1976) and as his assistant, Frémont was the logical choice to go in his stead. But if the data weren't actually needed for Nicollet's (1843a) map, then indeed one might ask why he was sent. Was the lower Des Moines River valley actually an unexplored lacuna? The facts suggest largely that it was not.

On 7 June 1835, Companies B, H, and I of the United States Regiment of Dragoons under the command of Lt. Col. Stephen Watts Kearny (1794–1848) departed Fort Des Moines, then situated on the Mississippi River at what is today Montrose in Lee Co., Iowa (Lea, 1836; van der Zee, 1914; Pelzer, 1917; Gourley, 2009). For the next 12 weeks, the dragoons explored much of what is today Iowa and southern Minnesota. Of particular interest here is the fact that Lt. Albert Miller Lea (1804–1891) descended the Des Moines River in a canoe, just as Frémont would do six years later. Like Frémont, he created a detailed map as he went (Lea, 1890; Pelzer, 1917). Lea's map and all accompanying notes and sketches were made available to Nicollet; Lea even met Nicollet in Washington in 1841 while serving as Chief Clerk of the War Department (Lea, 1890). Clearly, Jackson and Spence (1970: 96) erred when they stated that there "was no continuous and extensive survey of the entire river below the forks" prior to Frémont's 1841 survey.

Furthermore, the area of the Raccoon Forks already had been surveyed. In the summer of 1838, Capt. Nathan Boone (1781–1856) of the Dragoons and Capt. Augustus Canfield (1801–1854) of the Topographical Engineers charted the area carefully while laying out the course of a military road between Fort Leavenworth on the Missouri River and Fort Snelling at the mouth of the Minnesota River (Lees, 1914; Hurt, 1998).

Finally, another member of the Corps of Topographical Engineers, Capt. Walter Burling Guion (1808–1845), had just visited the same stretch of the Des Moines. His orders were issued by Col. Abert on 1 December 1840, six months before Frémont's orders, and the work was performed "so soon as favorable weather in the spring [of 1841] ... permitted" (Guion, 1842: 13). The resulting report (Guion, 1842), dealing with the bed of the river and impediments it presented to navigation, was dated 9 October 1841.

With the completion of these three surveys, the lower Des Moines River and the Raccoon Forks had already been surveyed as extensively as many other locations on Nicollet's (1843a) map. In light of this, the rationale for Abert's order to Frémont indeed is unclear.

Personnel and Preparations

The primary structure of the following two sections is a synthesis of Frémont's (1842) published report with official government documents unearthed in various archives and published verbatim by Jackson and Spence (1970). Consequently, those two sources are not cited again in this section, other than to substantiate direct quotes. This basic narrative is then fleshed out with facts from diverse secondary sources, which are cited. Assumptions, interpretations, and extrapolations originating with me are stated in first person for clarity.

Frémont arrived in St. Louis, Missouri, no later than Monday, 21 June 1841. He took lodging at B. W. Ayres' Green Tree Tavern at 68 Second St., and spent the next four days purchasing equipment and supplies. For example, at the hardware store of Adolphus Meier & Co. at 23 Main St., he bought cooking equipment, axes, and a tin lantern; from grocers Angelrodt, Eggers & Barth at 165 Main St., numerous expendables, including a half barrel of crackers, 50 lb. of rice, 25 lb. of coffee, 4 lb. each of tea and chocolate, 6 lb. of candles, 2 1/2 lb. of soap, and 34 lb. of chewing tobacco (the last presumably intended for barter with the Sauk and Meskwaki); and from optician Jacob Blattner, 34 Olive St., a $50 Bavarian-made spyglass. Frémont also bought a gray horse for $75, and fitted it out with a Spanish saddle, bridle, martingale, and three side hobbles.

While in St. Louis, Frémont sought out Pierre Chouteau, Jr. (1789–1865), whom he had met during the Nicollet expeditions. Chouteau was the proprietor of the trading house that once had been the Western Department of the American Fur Co. (Chittenden, 1902), a powerful and influential man throughout the West. As he had done for Nicollet, Chouteau provided Frémont with letters of introduction, directing his firm's representatives on the Des Moines River to assist the topographer in carrying out his survey.

Frémont also sought out another familiar face from the Nicollet expeditions: Karl Geyer. Though Geyer's name appears nowhere in Frémont's (1842) report, he later stated (Frémont, 1887: 68) that during the Des Moines River expedition, "Mr. Geyer did all that the season and time allowed for botany." Geyer was hired at the rate of $1.50 per diem, not as a botanist but as a boat hand (engagé), a subterfuge I suspect was necessitated by the War Department's lack of interest in botanical studies. Seeking to supplement this paltry salary, Frémont wrote to Nicollet on the botanist's behalf on 23 June (see below). Through Geyer, Frémont met Dr. George Engelmann (1809–1884), a local physician. Engelmann had botanized around St. Louis with Geyer the previous year (Reichenbach, 1855; McKelvey, 1955), and soon would become the foremost botanist of his day to reside in the West (White, 1896; Spaulding, 1909; Shaw, 1986; Wolf, 1988; Long, 1995).

Frémont also hired Clément Lambert (1807–1879), an experienced voyageur, as boat hand and cook. The following year, Lambert participated in Frémont's expedition to the Rocky Mountains (Frémont, 1845a, 1845b, 1846), and a decade after that, he became one of the founding fathers of Decatur, Nebraska (DeMilt, 1902; Sutherland, 1929).

Itinerary

To reach the Des Moines River from St. Louis, Frémont's party took passage on the *Monsoon*, a first-class packet boat that operated for many years on Western waters (Steele, 1841; Mahan, 1921); Capt. William S. Randolph was her master (Anonymous, 1841; Hughes, 1841). For the 1841 season, she steamed upriver from St. Louis to Keokuk in Iowa Territory every Monday and Friday at 4:00 PM, returning to St. Louis the following day at 8 PM. Her advertised ports-of-call (from south to north) were Alton, Illinois; Hannibal and Marion City, Missouri; Quincy and Warsaw, Illinois; and "all intermediate ports" (Anonymous, 1841).

Frémont, Geyer, and Lambert steamed away from St. Louis at 4:00 PM on Friday, 25 June. The young lieutenant took one of the gentlemen's cabins that surrounded the dining room at the center of the middle deck at a cost of $5, while his engagés had to make do with deck passage at $2 each. No mention is made of lading the horse he had purchased, unless it counted as one of the eight packages of merchandise for which he paid 75¢ in freight charges.

The next day (Saturday, 26 June), the trio disembarked at Churchville in Clark Co., Missouri. This tiny settlement (subsequently subsumed into the village of Alexandria; Anonymous, 1887) was directly across the Mississippi River from Warsaw, Illinois, and approximately five miles downstream from Keokuk, Iowa. In 1841, the confluence of the Des Moines River and the Mississippi lay just a few hundred yards above the settlement (cf. Judson, 1838), although today the river's mouth has meandered more than two miles farther north.

The three men left Churchville on Sunday, 27 June. Equipment and supplies were transported by L. B. Mitchell of Churchville in his mule-drawn wagon. He received $34.93 for transportation of the load "from Churchville, Mo., to the trading post of the American Fur Co. [the antecedent of Chouteau & Co.] in the Sac and Fox [Sauk and Meskwaki] Indian country," and an additional $20 for "transportation of two men [i.e., Geyer and Lambert] between same places" (Jackson & Spence, 1970: 110). I assume that Frémont rode the horse he had purchased; at one point, he mentions a ride of about 12 miles.

Departing Churchville, the group followed a northwesterly course between the Des Moines and Fox rivers. The first nine miles lay over the broad open floodplain of the Mississippi. South of St. Francisville, Missouri, they ascended to the uplands and continued along the ridge where today are found the villages of Peaksville and Revere, Missouri. At first, their route along this ridge ran through oaks. But after two miles, the trees gave way to prairie, through which they rode for all the next day (Monday, 28 June). At some point along this northwesterly trek, they left Missouri, and entered the territory of Iowa in Van Buren Co.

These first two days, the party covered more than 40 miles. That evening they reached "a farm house on the verge of the prairie, about two miles and a half from Chinquest [Chequest] creek" (Frémont 1842: 16). I deduce from the following facts that this farmhouse was in extreme southwestern Van Buren Township (Township 69 North, Range 10 West of the Fifth Principal Meridian), in section 31 or 32. Frémont wrote that the next day, they crossed Chequest Creek at a spot four miles above its mouth. However, he never mentioned crossing Indian Creek, a similar tributary that enters the Des Moines from the west farther downstream, which they would have had to cross at some point. From this, I infer that they continued west until Indian Creek was quite inconsequential, near or above its headwaters. A line drawn from this area up to a point on Chequest Creek four miles above its mouth would run due north, and 2 1/2 miles south of the creek puts them in section 31 or 32 of Van Buren Township. Old maps (Judson, 1838; Anonymous, 1878a) indeed show prairie giving way to forest in this area. According to the General Land Office (<www.glorecords.blm.gov/>), the following men held land patents in those sections at that time: Jerome Briggs, Aaron Harlan, Allan Hiatt, and Thomas Groome. Likely it was with one of these pioneer settlers that the survey party passed the night.

On Tuesday, 29 June, the party departed this farmhouse and headed north. Crossing Chequest Creek (see above), they continued on through broken wooded hills until they reached the Des Moines River at the village of Portland, known today as Leando (Savage, 2007). There they forded the river and continued upstream on the left bank. They passed through Iowaville, a then-important village of which no vestige remains (Evans, 1907), and crossed the line established by the treaty of 21 October 1837 (*7 Stat., 540*). Beyond this line, the territory of Iowa was closed to settlement; the only legal residents were government-licensed traders and the indigenous Sauk and Meskwaki.

Frémont and company continued up the left bank of the river, through the extreme northeastern corner of what would become Davis Co., entering the future Wapello Co. at its southeastern corner. That evening, having covered another 28 miles, they reached their initial destination: the trading post owned by Pierre Chouteau, Jr. & Co.

This establishment comprised eight buildings on the left bank of the Des Moines River, 87 miles above its mouth, at what is today the city of Ottumwa (Anonymous, 1878b; Waterman, 1914; Gallaher, 1916). On the opposite bank were the villages of the Sauk chief Keokuk (1767–1848), and the Meskwaki chiefs Wapello (1787–1842) and Appanoose (Carter, 1900; Ewan, 1930; Nagel, 2009). Three miles to the east, the War Department's Bureau of Indian Affairs maintained an agency (Anonymous, 1878b; Waterman, 1914; Nagel, 2009). At the time of Frémont's survey, Lt. John Beach (1812–1874) had recently succeeded his father-in-law, Gen. Joseph Montfort Street (1782–1840), as agent in charge.

The Chouteau & Co. post was supervised by William (Captain Billy) Phelps (1809–1889), a resident partner of the firm, who lived there with his wife Caroline Kelsey Phelps (1812–1861) and their children (Ewan, 1930; Nagel, 2009). Phelps was "a large swarthy man, as brave as a lion" (Hussey, 1900: 347) and "a professed infidel in sentiment" (Davidson, 1893: 37). With his brother Stephen Sumner Phelps (1805–1880), he had been engaged in trade at various places in west-central Illinois and along the Des Moines River since 1828 (Button, 1884; Hussey, 1900; Ewan, 1930). To the Native Americans with whom he dealt, he was Chechepequa (Winking Eye), a sobriquet occasioned by his penchant for practical jokes; Gov. Robert Lucas (1781–1853) once was the victim of a particularly elaborate prank (Anonymous, 1878b; Sabin & Sabin, 1900; Waterman, 1914). In anticipation of the westward relocation of the Sauk and Meskwaki (Anonymous, 1878b; Rogers, 1993), Phelps moved the company's post farther upriver the following year. With permission from John Sanford of Chouteau & Co., the abandoned buildings were occupied from 12 October 1842 to 17 May 1843 by Company I, First Regiment of Dragoons, under the command of Capt. James Allen (1806–1846). This temporary Army post was unofficially dubbed Fort Sanford (Carter, 1900; van der Zee 1913, 1914; Nagel, 2009), and its site is known to this day as Garrison Rock.

On Wednesday, 30 June, the hired rig returned to Churchville, while the survey crew made preparations. From Phelps, Frémont obtained considerable information on the river's suitability for navigation. The trader had brought the Chouteau & Co. steamboat *Pavillion* up the Des Moines on several occasions (Hussey, 1900; Wright & Young, 1915; Ewan, 1930) and so was quite familiar with such matters. Ample notes were also made (presumably by Geyer) on the characteristic trees in the vicinity, both on the bluffs and on the bottoms.

When the survey party resumed their upriver journey on Thursday morning, 1 July, they were provided by Phelps with a guide and other necessities. By that evening, the group had covered about 20 miles, reaching a second trading post at or near what is today the city of Eddyville. Frémont denominated this "the house of Mr. Jameson—another of the [Chouteau &] company's posts" (Frémont 1842: 16), but the identity of this Jameson is problematic. Accounts of the local fur trade (Thompson, 1850; Anonymous, 1878b; Button, 1884; Carter, 1900; Chittenden, 1902; Waterman, 1914; Gallaher, 1916; van der Zee, 1916; Ewan, 1930; Rogers, 1993) make no mention of anyone named Jameson, though they do indicate the presence at that time of three additional trading posts on the Des Moines River in what is now Wapello Co.

The first of these posts was owned by brothers William G. Ewing (?–1854) and George Washington Ewing (1803–1866) of Indiana, and sat three miles downstream from Phelps's post, at the mouth of Sugar Creek. Its manager during 1841–1842 was Mr. Hunt, "a gentleman of far more education, refinement and culture" than the average trader (Anonymous, 1878b: 364), but whose bookkeeping practices proved problematic (Thompson, 1850). The second post was situated approximately 20 miles upstream from Phelps, at the mouth of Muchankinock Creek adjacent to the village of Sauk chief Wishecomaque, and was owned by Jabish P. Eddy, the eponym of Eddyville.

It was the third post, a quarter of a mile downstream from Eddy's, which I interpret as the one reached on Friday evening by the survey party. This "small establishment" (Anonymous, 1878b: 364) represented an addition to the Chouteau & Co. license held by Phelps. Why Frémont (1842: 16) referred to this as "the house of Mr. Jameson" is unknown. In his table of distances (Frémont 1842: 19), he called it "Vessar's Trading House." Phelps at that time did have in his employ a man named Simpson (Captain Suggs) Vasser. This man figured prominently in Phelps's prank on Gov. Lucas (Anonymous, 1878b; Sabin & Sabin, 1900; Waterman, 1914), and an inkling of his character may be inferred from his parenthetical sobriquet (cf. Hooper, 1845).

Friday, 2 July, was spent at this upriver post, making necessary preparations. Four additional engagés were hired from among the Chouteau & Co. employees, making a party of six under Frémont's command. For a man named Cameron and his horse, Frémont was billed $20; A. Netherson cost $24 to hire; and a Sauk named Packesayso, $11. The fourth man was Simpson Vasser (see above), for whose "services as pilot" (Jackson & Spence, 1970: 111) Frémont paid $36. This larger salary for Vasser suggests to me that he was the guide supplied by Phelps the day before at the main post, who led Frémont and company upstream to this second post.

Saturday, 3 July, the survey of the river valley commenced in earnest. Frémont forded the river and ascended the right bank, presumably on horseback. Five were said to be in the canoe, transporting the provisions and instruments. [I assume that the sixth man accompanied Frémont overland. Geyer, as botanist, would have been the obvious candidate for this; if so, perhaps Cameron's horse was hired for his

benefit. Alternatively, Cameron rode his own horse, guiding Frémont, while Geyer went by canoe.]

The canoe's progress against the current was so slow that Frémont was able to join them at night, after examining the neighboring country during the day. Unfortunately, he provided little information on their itinerary during this period, beyond their departure from the upper Chouteau post on Saturday, 3 July, and their arrival at the Raccoon Fork on Friday, 9 July. Our only details in this regard are derived from Geyer's herbarium specimens (see below).

On Sunday, 4 July, specimen labels place them "10 miles above the upper Saki [Sauk] village." [I assume this refers to the village of the Sauk-Hocąk prophet Wabokieshiek (1794–1841), which lay 1 1/4 miles upstream from the upper Chouteau post (Anonymous, 1878b) and about six miles below the mouth of Cedar Creek. This would place them in what is today Mahaska Co., about six miles southwest of the city of Oskaloosa.]

Our next fix on their position is Wednesday, 7 July, when they were at the mouth of English Creek in what is today Marion Co., just north of the village of Harvey. This is only 17 miles upstream from their position on 4 July. [Covering just 17 miles of river in three days suggests that during this period, Frémont ranged rather far afield through the neighboring countryside. His comment (Frémont, 1842: 17) that "the Missouri dividing ridge [i.e., the Chariton River watershed] ... [was] not more than twelve miles distant" suggests that he rode at least that far from the Des Moines, as does his extensive discussion of the prairie uplands bordering Cedar Creek (see below).]

In contrast to this testudinal pace, only two days were required to cover the 66 miles separating English Creek and their destination. Once they reached the mouth of the Raccoon River in what is today the city of Des Moines in Polk Co., Frémont complied with his orders by determining that point's latitude and longitude via astronomical observation.

While at the Raccoon Fork, Geyer had a close brush with a venomous serpent, presumably *Crotalus horridus* Linnaeus (Viperidae). Frémont (1887: 20) related that the botanist "stooped down and grasped the clustered head of a low flowering plant. Under the broad leaves lay coiled a rattlesnake, close to his hand. Geyer escaped, but it gave him a spasm that made him dig his heels into the ground and jerk his arms nervously about as he threw off the shock. Always afterward he looked for snakes among his flowers ... There were many snakes along the river, and botany became a hazardous pursuit."

Once the position of the confluence had been determined, Frémont proceeded to make a survey of the river from there to its mouth. He meticulously recorded distances between various landmarks, which suggests to me that the entirety of this survey was made via canoe. However, he did not record the date of departure from the Raccoon Fork, nor any other dates during this descent. The sole surviving herbarium specimen from the period was collected on Saturday, 17 July, at the Red Rocks in what is today Marion Co. (cf. Anonymous, 1881; Lees, 1914; Rogers, 1993). I assume that it was rather late in this descent of the river that Geyer's botanical collections "suffered much by the filling of a canoe" (Geyer, 1845: 480), based on the small number of surviving specimens. [If the accident and loss had occurred earlier, the industrious botanist would have had time to replace the material lost with fresh collections.]

Once the group passed Portland (Leando), they were on the portion of the river that they had bypassed during the overland portion of their ascent: the Great Bend of the river in Van Buren Co., with its rapids and the mouth of Indian Creek; Sweet Home and St. Francisville in Clark Co., Missouri; and the mouth of Sugar Creek in Lee Co., Iowa. They arrived back in Churchville, Missouri, no later than Tuesday, 20 July, having covered the 160 miles from the Red Rocks in just three days. Most of the hired hands had disembarked when they reached Phelps's trading post. The one Chouteau engagé who did not was Packesayso. At Churchville on Wednesday, 21 July, he received an additional $15.75 for service as a boatman through 24 July. [A reasonable interpretation of this anachronism is that he was paid to return the borrowed canoe to Phelps.]

The steamboat *Monsoon* had experienced a fire and grounding during her return to St. Louis on 28 June (Hughes, 1841). However, the damage was soon repaired and she was back in service by the time Frémont and party returned from the Raccoon Fork. Her advertised schedule (Anonymous, 1841) called for her to depart Warsaw, Illinois, at 8:00 PM on Tuesday, 20 July, and Quincy, Illinois, at 8:00 AM on Wednesday, 21 July. [Based on Packesayso's pay voucher (see above), I infer that the party embarked at Churchville very early Wednesday. Frémont's voucher for the steamboat fare was dated at St. Louis on Thursday, 22 July.]

Back in St. Louis, Frémont found a letter waiting for him from Nicollet, a reply to his of 23 June. Dated 11 July, it reported that "everyone here ... asks for you, even at Mr. B[enton]'s, each time I go there. The young ladies [i.e., Jessie and her sisters] arrived the

day before yesterday [from a family wedding in Virginia] . . . Everything is fine, you are happily and impatiently awaited" (Jackson & Spence, 1970: 97). In response to Frémont's concern for Geyer's financial situation, Nicollet wrote that he had arranged for Chouteau to pay Geyer an additional $100 on Nicollet's personal account.

Using funds deposited earlier in the Bank of St. Louis, Frémont settled his account with Chouteau & Co. The various company-supplied provisions consumed and the hire of the four engagés totaled $236.35. In light of his payment to L. B. Mitchell (see above), it is not clear why Chouteau & Co. billed him $10 for transportation from the mouth of the Des Moines to the Sauk and Meskwaki village, or $23 for 18 days use of a mule and wagon. Frémont also settled accounts with Lambert and Geyer, paying them $60.50 and $46.50 for their services, respectively.

The specimens that Geyer had gathered were entrusted for identification to George Engelmann (see above). It was perhaps with an eye toward ameliorating his impecuniosity that Geyer spent the following year collecting 20 duplicate sets of 150 specimens each at St. Louis and Beardstown, Illinois, which were described by Engelmann (1843) and offered for sale at $6 per set by Gray (1843).

Frémont was back in Washington no later than 12 August. If indeed the young lieutenant had been sent away to cool Jessie Benton's ardor for him, it was to no avail. On 19 October, while Sen. Benton was out of the city, the couple was wed secretly at the home of former Attorney General John Jordan Crittenden (1787–1863) by Father Joseph Van Horseigh of St. Peter's Roman Catholic Church (Frémont, 1887; Phillips, 1935).

Frémont's final report to Col. Abert, prepared in Jessie's handwriting, was dated 19 April 1842. He then was absent from Washington on his first expedition to the Rocky Mountains from 2 May to 29 October (Frémont, 1843; Welsh, 1998). On 10 December, he replied to the query about the Des Moines River survey from Rep. Edwards (see above), and that reply and his report (along with Capt. Guion's report; see above) were published by the United States House of Representatives on 31 December 1842. Despite Sen. Benton's supposed interest in the expedition and its bearing on the Iowa–Missouri border (see above), there was no Senate version of the report (cf. Nicollet, 1843b, 1845; Torrey, 1843, 1845).

Descriptions of Vegetation

In closing his report to Col. Abert, Frémont wrote that the "botany and geology of the region visited occupied a considerable share of my attention. Should it be required by the bureau, these may form the subject of a separate report" (Frémont, 1842: 18). The Bureau of the Corps of Topographical Engineers apparently saw no need for such a report, as none has been found in its archives (Jackson & Spence, 1970).

However, the report that was submitted and published did contain narrative descriptions of the vegetation in various areas. The plant communities Frémont described constituted both prairie and forest, with upland and bottomland representations of each (cf. Roosa et al., 1989; Eilers & Roosa, 1994; Yatskievych, 1999). Earlier works on Iowa (e.g., Plumbe, 1839; Galland, 1840) had contained descriptions of this sort, but Frémont's report differed in the use of binomials instead of vernacular names. For example, compare Galland's (1840: 8) simple reference to Iowa possessing "all the varieties of oak" with Frémont's (1842) careful notes on the ecological and geographical distribution of the various species of *Quercus* L. It is this commitment to the formalities of botany and the participation of a professional botanist (Geyer) that make Frémont's report the first scientific publication devoted to the Iowa flora.

Unfortunately, many of the binomials were garbled badly in print. According to Jackson and Spence (1970), this was not true with the original handwritten report. From this I infer that the errors originated with a typesetter unschooled in botany, and that Frémont did not have the opportunity to review page proofs. In the present section, I have corrected these misspellings; the original garbled version will be found in the next section, where the individual species and their authors are listed.

Upland Forest

In Clark Co., Missouri, the southeastern end of the ridge between the Des Moines and Fox rivers was dominated by blackjack oak (*Quercus marilandica*) and shingle oak (*Q. imbricaria*). The predominance of these species suggests a relatively dry open forest (or perhaps even savannah) on this ridge. Farther upriver at Phelps's trading post, the bluffs along the river were dominated by white oak (*Q. alba*), mixed with bur oak (*Q. macrocarpa*), black oak (*Q. velutina*), and shagbark hickory (*Carya ovata*). These are the typical species in most of Iowa's forest on dry to mesic uplands.

Between the Cedar Creek prairie (see below) and the Raccoon Fork, "the whole region [was] densely and luxuriantly timbered . . . covered with heavy and dense bodies of timber, with a luxuriant soil and almost impenetrable undergrowth. . . . The heaviest bodies lie on the Three Rivers [in what is today

northeastern Warren Co.], where it extends out to the top of the main ridge, about thirty miles. On the northern side of the Des Moines, the ridge appeared to be continuously wooded" (Frémont 1842: 17). Both bluffs and level uplands in this region were dominated by sugar maple (*Acer saccharum* Marshall), black walnut (*Juglans nigra*), butternut (*J. cinerea*), and hackberry (*Celtis occidentalis*), indicating an even more mesic setting.

BOTTOMLAND FOREST

Forests on floodplains and lower terraces differed considerably in their composition. Around Phelps's trading post, they comprised basswood (*Tilia americana*), American elm (*Ulmus americana*), slippery elm (*U. rubra*), river birch (*Betula nigra*), pecan (*Carya illinoinensis*), hop-hornbeam (*Ostrya virginiana*), Kentucky coffee-tree (*Gymnocladus dioicus*), and hackberry (*Celtis occidentalis*). In areas increasingly prone to inundation, silver maple (*Acer saccharinum* L.), cottonwood (*Populus deltoides*), and groves of sandbar willow (*Salix interior*) dominated.

BOTTOMLAND PRAIRIE

Along the lower course of the river, the floodplains and terraces often were not forested. The floodplain of the Mississippi between the Des Moines and Fox rivers in Clark Co., Missouri, was "a luxuriant prairie bottom . . . covered with a profusion of flowers, among which the characteristic plant was *Psoralea onobrychis*" (Frémont, 1842: 16). On the left bank of the Des Moines River in Van Buren Co., Iowa, a few miles above Leando where the floodplain averages one-quarter to one-half mile in width (Lees, 1914), Frémont noted "quantities of *Liatris resinosa* [Nutt.], mingled with *Rudbeckia digitata* [Mill.]" (Frémont, 1842: 16); such species are likewise characteristic of bottomland prairie. He also mentioned an unspecified location where "prairie bottoms bordering the river were filled with *Liatris pycnostachya* [Michx.]" (Frémont, 1842: 16).

UPLAND PRAIRIE

The highest ridges and the broad level areas between streams were also clad in prairie. The dry open oak forest on the ridge between the Des Moines and Fox rivers (see above) gave way after two miles to "a narrow level prairie . . . from one and a half miles to three miles in width, limited by the timber which generally commences with the descent of the river hills" (Frémont, 1842: 16). Here, the *Psoralea onobrychis* that had been so common in the bottomland prairie "gave place . . . to a species of *Cacalia* [L.], which was followed, on its disappearance further up, by *Parthenium integrifolium*" (Frémont, 1842: 16). This prairie extended from west of St. Francisville, Missouri, to within 2 1/2 miles of Chequest Creek in Van Buren Co., Iowa. Frémont also mentioned a narrow prairie between Chequest Creek and Soap Creek. The latter stream enters the Des Moines from the west upstream from Iowaville, so that this prairie would have begun in northwestern Van Buren Co. and extended westward into what is today Davis Co., Iowa.

The most expansive prairie seen by Frémont lay in what is today Mahaska, Marion, and Monroe counties, Iowa. This prairie began at the mouth of Cedar Creek, a tributary that enters the Des Moines from the west in southwestern Mahaska Co. It covered all the uplands from there south and west to the ridge marking the watershed of the Chariton River, a tributary of the Missouri River. It was described as having "a rich soil, covered with the usual innumerable flowers and copses of hazel and wild plum" (Frémont: 1842: 17).

List of Species

The specimens that Geyer collected and gave to George Engelmann (see above) today reside with the remainder of Engelmann's collections in the herbarium (MO) of the Missouri Botanical Garden (Wolf, 1988). Seven specimens from the 1841 Frémont–Geyer expedition appear on that institution's Tropicos® database (<http://www.tropicos.org/>). Curiously, none of them represent species mentioned in print; the two sets of names are mutually exclusive.

I visited MO in March 2013 seeking vouchers for species mentioned in the report, but found not one. I also searched the herbarium for any 1841 Geyer specimens of species that might be expected to bloom in early summer in the Des Moines River valley, but likewise with no success. It indeed appears that most of Geyer's specimens collected in Iowa were destroyed in the canoe accident on the river (Geyer, 1845). Geyer (1845) believed that the survivors included new taxa, but I am unaware of any name, effectively published or otherwise, that was based on these specimens (Lammers, 1985; Wolf, 1988).

In this section, these seven documented species are intercalated with the 30 species mentioned in the report. They are arranged alphabetically rather than by family, to facilitate intercalation with Welsh's (1998) list of species from Frémont's subsequent expeditions. For each specimen, label data are quoted verbatim (portions in brackets are editorial additions) and its page on Tropicos® cited. For each

literature report, information presented in quotation marks is verbatim (Frémont, 1842: 16–17); the remainder is my editorial interpretation. Information on the geographic distribution of species in the region derives from Roosa et al. (1989), Eilers and Roosa (1994), and Yatskievych (1999, 2006, 2013) unless otherwise indicated.

As noted in the preceding section, the names used by Frémont (1842) were often garbled by the typesetter. Aside from these orthographic errors, the names employed in several cases still differ from those used to denote the same entities today. See Lammers (2013) for an extended discussion of how I have dealt with this problem of synonymy in early literature.

Acer saccharinum L. (Sapindaceae) – "Acer eridcarfurm" was observed along the Des Moines River from the Raccoon Fork to its mouth, "on the banks of such rivers as have a gravelly bed . . . seen almost constantly along the shore." *Acer eriocarpum* Michx. is a heterotypic synonym of *A. saccharinum* (Torrey & Gray, 1840), which is common throughout Iowa.

Acer saccharum Marsh. (Sapindaceae) – "Acer saccharium" was observed along the Des Moines River below Raccoon Fork, in what is today Polk or Warren Co., Iowa, in "heavy and dense bodies of timber . . . on the broken slopes of the bluffs as on the uplands." In this part of Iowa, trees of *A. saccharum* subsp. *nigrum* (Michx. f.) Desmarais tend to be more common than those of the autonymic subspecies, though both occur.

Actaea rubra (Aiton) Willd. (Ranunculaceae) – IOWA. [**Marion Co.:**] shady rocky woods of the upper Des Moines at the mouth of English River [i.e., English Creek], 7 July 1841, *C. A. Geyer s.n.* (MO) [<http://www.tropicos.org/Specimen/2605002>]. The specimen was identified by J. M. Kane in 1966. This forest herb is rare in southeastern and south-central Iowa though common elsewhere in the state.

Arnoglossum plantagineum Raf. (Asteraceae) – "A species of causalia" [*Cacalia* L., nom. rej.] was seen to be "very abundant" west and northwest of St. Francisville in Clark Co., Missouri, on the southerly portion of the "narrow level prairie, occupying the summit of the ridge between the Fox and Des Moines rivers." Midwestern species formerly placed in *Cacalia* now are assigned to either *Arnoglossum* Raf. or *Hasteola* Raf. Of the species known from northeastern Missouri, only *A. plantagineum* would be found on upland prairie (Yatskievych, 2006).

Betula nigra L. (Betulaceae) – "Betula rubra" was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps . . . on the bottom land of the creeks" in what is today Ottumwa in Wapello Co., Iowa. *Betula rubra* Michx. f. is a heterotypic synonym of *B. nigra* (Furlow, 1997), which is common in eastern Iowa but less so toward the southwestern counties.

Calamagrostis canadensis (Michx.) P. Beauv. (Poaceae) – IOWA. [**Warren Co.:**] near the mouth of the two rivers on the upper Des Moines River [i.e., Middle River and South River, northeast of Hartford], in poplar thickets on a sandbar, 9 July 1841, *C. A. Geyer s.n.* (MO) [<http://www.tropicos.org/Specimen/2501424>]. This wetland grass is frequent to common throughout much of Iowa.

Carya illinoinensis (Wang.) K. Koch (Juglandaceae) – "Carya olivaeformis" was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps . . . on the bottom land of the creeks" in what is today Ottumwa in Wapello Co., Iowa. *Carya oliviformis* (Michx.) Nutt. is a heterotypic synonym of *C. illinoinensis* (Stone, 1997). Pecan trees are very rare in Iowa, known only from the floodplain of the Mississippi River in the southeastern and east-central counties (Lammers & van der Valk, 1977); this is the sole report of them from anywhere else in the state.

Carya ovata (Mill.) K. Koch (Juglandaceae) – "Carya alba" was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps . . . [o]n the bluffs" and "now and then . . . in the bottoms" in what is today Ottumwa in Wapello Co., Iowa. *Carya alba* (L.) Nutt., nom. rej., was applied to both *C. ovata* and *C. tomentosa* (Poir.) Nutt. (Stone, 1997); the former is by far the more common of the two in Iowa.

Ceanothus americanus L. (Rhamnaceae) – "Clanothus" [*Ceanothus* L.] was observed in the "river hills, which were broken, wooded, and filled with [its] delicate fragrance" south of Chequest Creek and four miles above its mouth in Van Buren Co., Iowa. Of the two species of the genus in Iowa, *C. americanus* is by far the more common in the southeastern counties, particularly in forested settings.

Celtis occidentalis L. (Cannabaceae) – "Celtis accidentalis" was "found everywhere" on the left bank of the Des Moines River "at the post of Mr. Phelps" in what is today Ottumwa in Wapello Co., Iowa. *Celtis crassifolia* Lam. was observed on the Des Moines River below the mouth of the Raccoon River in Polk Co. or Warren Co., in "heavy and dense bodies of timber . . . on the broken slopes of the bluffs as on the uplands." This name is a heterotypic synonym of *C. occidentalis* (Fernald, 1950), which is common throughout Iowa.

Clematis pitcheri Torr. & A. Gray (Ranunculaceae) – IOWA. [**Van Buren** or **Davis Co.:**] Banks of Des Moines River near Iowaville, 29 June 1841, *C. A.*

Geyer s.n. (MO) [<http://www.tropicos.org/Specimen/100241782>]. The specimen was identified by R. O. Erickson in 1942. Because *C. pitcheri* had been described only a few years earlier (Torrey & Gray, 1838), this may be one of the specimens that Geyer (1845) thought represented an undescribed species. It is common in southeastern Iowa, but less so elsewhere in the state.

Corylus americana Walt. (Betulaceae) – "Hazel" was observed "on the uplands bordering Cedar river" [i.e., Cedar Creek] in what is today Mahaska Co., Iowa, in "prairie with a rich soil." This shrub is common throughout most of Iowa.

Eragrostis pectinacea (Michx.) Nees (Poaceae) – IOWA. [**Marion Co.:**] on a sandbar of the upper Des Moines near mouth of English River [i.e., English Creek], 7 July 1841, *C. A. Geyer s.n.* (MO) [<http://www.tropicos.org/Specimen/2560310>]. The specimen was identified by L. H. Harvey in 1981. This annual grass is common throughout the state.

Festuca paradoxa Desv. (Poaceae) – IOWA. [**Marion Co.:**] grassy low banks of the upper Des Moines River at the mouth of English River [i.e., English Creek], 7 July 1841, *C. A. Geyer s.n.* (MO) [<http://www.tropicos.org/Specimen/2573714>. The specimen was identified by C. A. Taylor in 1958. Because *F. paradoxa* had been described just 10 years earlier in an obscure European journal (Desvaux, 1831), this may have been one of the specimens thought by Geyer (1845) to represent an undescribed species. It is very rare in Iowa, found at scattered localities in the south-central and east-central counties (Pohl, 1966).

Gymnocladus dioicus (L.) K. Koch (Fabaceae) – "Gymnoeladus canadensis" was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps . . . on the bottom land of the creeks" in what is today Ottumwa in Wapello Co., Iowa. *Gymnocladus canadensis* Lam., nom. illeg, is a homotypic synonym of *G. dioicus* (Yatskievych, 2006), which is frequent to infrequent throughout Iowa.

Juglans cinerea L. (Juglandaceae) – "Inglans cathartica" was reported from the Des Moines River below the mouth of the Raccoon River in Polk Co. or Warren Co., in "heavy and dense bodies of timber . . . on the broken slopes of the bluffs as on the uplands." *Juglans cathartica* Michx. is a heterotypic synonym of *J. cinerea* (Nuttall, 1818), which is common in the eastern half of Iowa, but less so toward the southwestern counties.

Juglans nigra L. (Juglandaceae) – "Inglans nigra" was reported from the Des Moines River below the mouth of the Raccoon River in Polk Co. or Warren Co., in "heavy and dense bodies of timber . . . on the broken slopes of the bluffs as on the uplands." This tree is common throughout Iowa.

Juncus tenuis Willd. (Juncaceae) – IOWA. [**Mahaska Co.:**] on a sandbar of the upper Des Moines, 10 mi. above the upper Saki [Sauk] village, 4 July 1841, *C. A. Geyer s.n.* (MO) [<http://www.tropicos.org/Specimen/100314746>]. After the binomial is written "var" followed by a four-word Latin ablative phrase; perhaps this is one of the specimens that Geyer (1845) thought represented an undescribed taxon. This rush is common throughout most of Iowa.

Liatris aspera Michx. (Asteraceae) – "Lyatris resinosa" was observed "a few miles above Portland [i.e., Leando], on the north bank of the river" in Van Buren Co., Iowa. *Liatris resinosa* Nutt. is a homotypic synonym of *L. spicata* (L.) Willd. var. *resinosa* (Nutt.) Gaiser, a taxon found only in the southern United States (Nesom, 2006). Of the species of this genus occurring in Iowa, *L. aspera* most likely was the plant Frémont saw, as it is frequent to common throughout the state and resembles *L. spicata* in inflorescence structure.

Liatris pycnostachya Michx. (Asteraceae) – "Lyatris pycnostachya" was seen in "prairie bottoms bordering the river" in Van Buren Co., Iowa. This perennial herb is frequent to common throughout most of Iowa.

Ostrya virginiana (Mill.) K. Koch (Betulaceae) – "Osteya Virginica" was seen on the left bank of the Des Moines River, "at the post of Mr. Phelps . . . on the bottom land of the creeks" in what is today Ottumwa in Wapello Co., Iowa. This small hardwood is frequent to common throughout the state.

Parthenium integrifolium L. (Asteraceae) – This species was seen to be "very abundant" west and northwest of St. Francisville in Clark Co., Missouri, on the northerly portion of the "narrow level prairie, occupying the summit of the ridge between the Fox and Des Moines rivers." This perennial herb is found throughout most of Missouri (Yatskievych, 2006).

Polygala senega L. (Polygalaceae) – IOWA. [**Marion Co.:**] on the top of the Red bluffs, Upper Des Moines River, 17 July 1841, *C. A. Geyer s.n.* (MO) [<http://www.tropicos.org/Specimen/1055872>]. The specimen was identified by S. K. Chen in 1988 and A. E. Trauth in 1997. This perennial herb is rare in central and southeastern Iowa, becoming more frequent toward the northeastern counties.

Populus deltoides Bartram ex Marsh. (Salicaceae) – *Populus canadensis* Moench was reported from the left bank of the Des Moines River, "at the post of Mr. Phelps . . . form[ing] groves in the inundated river bottoms" in what is today Ottumwa in Wapello Co., Iowa. This binomial has been misapplied to *P.*

deltoides, a common tree throughout Iowa; the name was actually based upon a hybrid between North American *P. deltoides* and Eurasian *P. nigra*, which occurred in cultivation in Europe (Argus et al., 2010).

Prunus americana Marsh. (Rosaceae) – "Wild plum" was observed "on the uplands bordering Cedar river [i.e., Cedar Creek]" in what is today Mahaska Co., Iowa, in "prairie with a rich soil." This is the common native plum throughout Iowa.

Psoralea onobrychis Nutt. (Fabaceae) – Frémont declared that "psoralia onolaychis" was a "characteristic plant" of the "luxuriant prairie bottom, bordered by the timber of the Fox and Des Moines rivers" on the Mississippi River floodplain "near the mouth of the Des Moines" in Clark Co., Missouri. This perennial legume is rare in Missouri, known only from the northeastern corner south to Franklin and Osage counties; in Iowa, it is found only in the extreme southeastern counties (Roosa et al., 1989; Isely, 1998).

Quercus alba L. (Fagaceae) – White oak was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps" in what is today Ottumwa in Wapello Co., Iowa, "[o]n the bluffs" and "now and then ... in the bottoms." It is very common throughout Iowa.

Quercus imbricaria Michx. (Fagaceae) – Shingle oak was observed on the bluffs overlooking the Mississippi River floodplain south of St. Francisville in Clark Co., Missouri, between the Des Moines and Fox rivers. It is common throughout most of Missouri (Yatskievych, 2013).

Quercus macrocarpa Michx. (Fagaceae) – "Quercus malrocarpa" was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps" in what is today Ottumwa in Wapello Co., Iowa, "[o]n the bluffs" and "now and then ... in the bottoms." Bur oak is frequent to common throughout Iowa.

Quercus marilandica Muenchh. (Fagaceae) – *Quercus nigra* L. was reported from the bluffs overlooking the Mississippi River floodplain south of St. Francisville, between the Des Moines and Fox rivers in Clark Co., Missouri. However, that species that does not ascend the Mississippi River farther than southeastern Missouri and inhabits wet woodlands not bluffs (Nixon, 1997). Of the oaks found in northeastern portion of the state (Yatskievych, 2013), *Q. marilandica* most likely was the one seen.

Quercus velutina Lam. (Fagaceae) – "Quercus tuictoria" was reported from the left bank of the Des Moines River, "at the post of Mr. Phelps" in what is today Ottumwa in Wapello Co., Iowa, "[o]n the bluffs" and "now and then ... in the bottoms." *Quercus tinctoria* W. Bartram is a heterotypic synonym of *Q. velutina* (Nixon, 1997), which is frequent to common throughout much of the state.

Rudbeckia laciniata L. (Asteraceae) – "Rudbackia digetata" was reported from "a few miles above Portland [i.e., Leando], on the north bank of the river" in Van Buren Co., Iowa. *Rudbeckia digitata* Mill. is a heterotypic synonym of *R. laciniata* var. *humilis* A. Gray, a taxon known only from the southeastern United States (Urbatsch & Cox, 2006). Iowa populations of *R. laciniata* belong to the autonymic variety.

Salix interior Rowlee (Salicaceae) – "Salex" [*Salix* L.] was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps" in what is today Ottumwa in Wapello Co., Iowa, "form[ing] groves in the inundated river bottoms." Of the several species of willow in Iowa, *S. interior*, which is common throughout, is most likely to form groves in the indicated habitat.

Sambucus canadensis L. (Caprifoliaceae) – "Elder ... fragrant and white" was reported from the left bank of the Des Moines River above Portland [i.e., Leando] in Van Buren Co., Iowa. It is common throughout Iowa.

Tilia americana L. (Tiliaceae) – *Tilia* L. was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps" in what is today Ottumwa in Wapello Co., Iowa, "in the bottoms." *Tilia americana* is the only native species of the genus in Iowa and it is common throughout.

Ulmus americana L. (Ulmaceae) – "Ulmes Americana" was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps" in what is today Ottumwa in Wapello Co., Iowa, "on the bottom land of the creeks." This tree was common throughout Iowa prior to 1928, when pathogenic fungi of the genus *Ophiostoma* Syd. & P. Syd. (Ascomycota) were introduced to North America.

Ulmus rubra Muhl. (Ulmaceae) – "Ulmes fulvia" was observed on the left bank of the Des Moines River, "at the post of Mr. Phelps" in what is today Ottumwa in Wapello Co., Iowa, "on the bottom land of the creeks." *Ulmus fulva* Michx. is a heterotypic synonym of *U. rubra* (Sherman-Broyles et al., 1997), which is frequent to common throughout much of Iowa.

Summary

The 1841 Frémont–Geyer expedition to the Des Moines River left to posterity information on 37 species of flowering plants. Seven herbaceous species are documented by Geyer's specimens at MO; one of these, *Festuca paradoxa*, today is rather rare in Iowa. The remaining 30 are unvouchered literature reports

(Frémont, 1842), representing 24 trees and shrubs of the forest and six herbs of the prairie. Of these, *Psoralea onobrychis* is rather rare in the region, while *Carya illinoinensis* never has been documented from the Des Moines River valley, only the Mississippi. Three (*Liatris spicata* var. *resinosa*, *Quercus nigra*, and *Rudbeckia laciniata* var. *humilis*) do not occur anywhere near the region and a more likely identity has been suggested for each.

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A MULTIVARIATE MORPHOMETRIC ANALYSIS OF THE *SOLIDAGO CHILENSIS* GROUP IN SOUTH AMERICA AND RELATED TAXA IN NORTH AMERICA (ASTERACEAE, ASTEREAE)[1]

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ABSTRACT

A multivariate morphometric study of *Solidago* L. in South America was undertaken to assess the numbers and ranks of taxa that could be usefully recognized. The results of stepwise discriminant, classificatory, and canonical analyses on a matrix of 50 traits of 160 specimens indicated the distinctiveness of the *S. chilensis* group of taxa from three morphologically similar North American species of the large *Solidago* subsect. *Triplinerviae* (Torr. & A. Gray) G. L. Nesom: *S. juliae* G. L. Nesom, *S. leavenworthii* Torr. & A. Gray, and *S. tortifolia* Elliott. Within the South American complex two species with predominantly allopatric distributions were statistically supported: *S. chilensis* Meyen and *S. microglossa* DC. were distinguished a priori on stem hair length. The cytogeography of the two species was investigated and all samples were diploid, $2n = 18$; *S. chilensis* (20 individuals from Argentina and one from Chile) and *S. microglossa* (six individuals from Argentina). Also, two other species of *Solidago* in South America that are not members of subsection *Triplinerivae* were found to have been misidentified as members of the *S. chilensis* complex; European *S. virgaurea* L. is a member of *Solidago* subsect. *Solidago* and native *S. argentinensis* Lopez Laphitz & Semple is a member of *Solidago* subsect. *Junceae* (Rydb.) G. L. Nesom. The name *S. chilensis* is neotypified.

Key words: Asteraceae, biogeography, cytogeography, multivariate morphometrics, *Solidago*.

The goldenrod genus *Solidago* L. (Asteraceae, Astereae) includes some 120 species primarily native to North America with a few species native to South America and a half dozen or more native to Eurasia (Semple, 2015). Semple and Cook (2006) recognized 77 species native to Canada and the United States, but more recent work indicates that as many as 103 species occur in the two countries (see Semple, 2015). The genus is divided into 13 subgeneric groupings with the taxonomically challenging *Solidago* subsect. *Triplinerviae* (Torr. & A. Gray) Nesom being the largest, with some 16 species. Members of subsection *Triplinerviae* are distinguished by (1) the absence of basal rosettes, (2) by having lower stem leaves that nearly always wither by flowering and that are not as large as lower mid-stem leaves, and (3) by having lower and mid-stem and sometimes upper stem leaves that are 3-nerved due to two lateral nerves being prominent (Semple & Cook, 2006). Heads are usually arranged in secund pyramidal inflorescence arrays. Pappus bristles are usually in two series that are sometimes similar, with the longer inner series usually weakly if at all clavate or rarely moderately so (Hood & Semple, 2003).

Multivariate studies on three sets of species in *Solidago* subsect. *Triplinerviae* have been investigated recently. *Solidago brendiae* Semple, *S. canadensis* L., *S. elongata* Nutt., *S. fallax* (Fern.) Semple, and *S. lepida* DC. were examined in one study (Semple et al., 2013), and *S. altiplanities* C. E. S. & R. John Taylor, *S. altissima* L., *S. canadensis*, and *S. juliae* G. L. Nesom were examined in a second study (Semple et al., 2015). Lopez Laphitz (2009) investigated the South American goldenrods in the *S. chilensis*/*S. microglossa* group and compared them to the morphologically similar North American species *S. juliae*, *S. leavenworthii* Torr. & A. Gray, and *S. tortifolia* Elliott of the informal Tortifolia group of *Solidago* subsect. *Triplinerviae* (Nesom, 1994; Semple, 2015). The results of this latter multivariate study are presented here.

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Lopez Laphitz et al. (2011) presented evidence that some South American collections identified as *Solidago chilensis* in the broad sense were in fact members of *Solidago* subsect. *Junceae* (Rydb.) G. L. Nesom and belonged in a new species, *S. argentinensis* Lopez Laphitz & Semple, closely related to the western North American *S. missouriensis* Nutt. Lopez Laphitz (2009) also noted that an Argentine specimen identified as *S. patagonica* Phil. was an escaped cultivar of *S. virgaurea*, a species native to Europe; *Cabrera 5015* (LP) was annotated as *S. virgaurea* by J. C. Semple in 2009. Sancho and Viera Barreto (2014) included this observation in their treatment of *Solidago* in Flora Argentina.

The number of ranks of taxa recognized in the literature on *Solidago* in South America has varied over time. Some 29 basionyms based on South American collections have been proposed, the majority for plants from Chile and Brazil (Hooker & Jackson, 1895, and supplements). Cabrera (1970) proposed the combination *S. chilensis* var. *megapotamica* (DC.) Cabrera and listed *S. microglossa* DC. as a synonym. Cabrera (1971) recognized two species, *S. chilensis* with densely headed inflorescences and *S. patagonica* sensu Cabrera with a sparsely headed inflorescence, in his treatment of *Solidago* in the Flora of Patagonia, basing the latter on *Cabrera 5015* (LP) by using head density to separate the two species. Cabrera (1974) recognized the one species *S. chilensis* with two varieties, glabrous variety *chilensis* and pubescent variety *megapotamica*, in his treatment of *Solidago* in the Flora Ilustrada Entre Rios (Argentina). Following Cabrera (1974), Sancho and Espinar (2003) recognized the same two varieties of *S. chilensis* in Flora Fanerogámica Argentina, again separating glabrous variety *chilenis* from pubescent variety *megapotamica*. Most recently, Sancho and Viera Barreto (2014) recognized three species of *Solidago* in Flora Argentina: *S. argentinensis*, *S. chilenis* (with variety *chilensis* and variety *megapotamica*), and *S. virgaurea* (based on *Cabrera 5015*, LP).

Kress et al. (2005) stated that genetic barriers among species of *Solidago* are poorly developed, and, consequently, a phylogeny of the genus had been difficult to obtain. While Brouillet et al. (2009) were successful in resolving the phylogeny of the tribe Astereae using ITS, Schilling et al. (2008) reported on a lack of resolution in the phylogeny of *Solidago* based on sequence data due to very low levels of variation in ITS and ETS. They concluded that the restriction fragment length polymorphisms based phylogenies in Zhang (1996; one phylogeny reproduced in Semple et al., 1999) were the only informative molecular phylogenies for the genus. No South American species of *Solidago* were included in Zhang (1996). Until more phylogenetically informative sequences are discovered in *Solidago*, alternative nonmolecular methods must be utilized to assess species limits within the genus.

Taxonomic treatments on *Solidago* in South America were based mainly on floristic descriptions only, and no multivariate study has ever been conducted on *S. chilensis* s.l. In order to determine the number of taxa in the complex and to assess the distinctiveness of members of the South American group from North American relatives, a multivariate morphometric analysis was undertaken. Because *S. chilensis* s.l. had been included in *Solidago* subsect. *Triplinerviae* at the time this study was initiated in 2007, three morphologically similar North American species of this subsection were included in the analysis for comparison.

The names of taxa used in this report are those accepted at the conclusion of this study.

Methods

Specimens

A total of 552 herbarium specimens, including those in WAT and specimens borrowed from other herbaria in the United States, Argentina, and Madeira (AAH, BRIT, GH, LL, LP, MADM, MO, and TEX), were examined. Of these, 350 herbarium specimens of *Solidago chilensis* s.l. were examined. Additionally, 52 specimens collected during a field trip through Argentina and Chile during May 2008 were included in the investigation. From all of these specimens, 118 specimens of *S. chilensis* and *S. microglossa* were selected for inclusion in the multivariate analyses based on the stage of floral development and completeness of the collection, i.e., those including at least mid- and upper stem and leaves and heads in anthesis. Using traits included in other analyses on the genus, descriptions of the taxa found in the literature (Semple & Cook, 2006), and personal observations, a list of the potentially useful and distinguishing traits was created (Table 1). Fifty morphological and floral traits were scored on these specimens. A 160-sample matrix of South American and related North American specimens was ultimately divided into five a priori groups; the numbers of individuals in each species-level a priori group were *S. tortifolia* (15), *S. juliae* (11), *S. leavenworthii* (15), *S. chilensis* (88), and *S. microglossa* (31). Location information on all South American collections examined was included in Lopez Laphitz (2009). Location information on North American collections is available on request from J. C. Semple.

Table 1. Traits characterized and measured for *Solidago chilensis* Meyen, *S. microglossa* DC., and related taxa from North America (*S. leavenworthii* Torr. & A. Gray, *S. juliae* G. L. Nesom, *S. tortifolia* Elliott). Traits with an asterisk (*) were not included in discriminant analyses because they correlated strongly (correlation coefficient of $|r| > 0.7$) with another trait or there were not enough measurements to be considered representative. Traits marked with the # symbol were also excluded as they were used to identify a priori groups in some analyses. Character codes are followed by their description.

Character code	Description
STEMHT*	stem height (cm)
ISTPUBD	stem hairs—number of hairs counted on 1 mm on inflorescence stem
USTPUBD*	stem hairs—number of hairs counted on 1 mm on upper vegetative stem
MSTPUBD*	stem hairs—number of hairs counted on 1 mm on midstem
BSTPUBD	stem hairs—number of hairs counted on 1 mm on basal stem
ISTHLN	hair length (mm) measured on inflorescence stem
USTHLN*	hair length (mm) measured on upper vegetative stem
MSTHLN*	hair length (mm) measured at midstem
BSTHLN*	hair length (mm) measured at basal stem
BLLENG*	basal leaf length (cm) measured from leaf base to apex
BLWID*	basal leaf width (cm) measured at widest point of blade
BLWTOE*	basal leaf (cm) measured from widest point to blade apex
BLSERAT*	basal leaf dentation counted as # of serrations on 1/2 of leaf margin
MLLENG	midstem leaf length (mm) as measured from leaf base to apex
MLWID	midstem leaf width (cm) as measured from widest point to blade apex
MLWTOE*	midstem leaf (mm) as measured from widest point to blade apex
MLSERAT#	midstem leaf dentation—number of serrations on 1/2 of margin
ULLENG	upper stem leaf length (mm) measured from leaf base to tip
ULWID	upper stem leaf width (mm) measured at widest point
ULWTOE*	upper stem leaf measured from widest point to tip (mm)
ULSERAT#	upper stem leaf dentation—number of serrations on 1/2 of leaf margin
ILLENG	inflorescence leaf length (mm) measured from leaf base to tip
ILWID	inflorescence leaf width (mm) measured at widest point
ILWTOE*	inflorescence leaf measured from widest point to tip (mm)
ILSERAT#	inflorescence leaf dentation—number of serrations on 1/2 of leaf margin
CAPBRANG	angle formed between main stem and branch measured from inflorescence
CAPW	width of inflorescence measured at widest point (cm)
CAPL	length of inflorescence measured from base to tip (cm)
LONGBR	length of longest branch in inflorescence (cm)
INVOLHT*	height of involucre (cm)
OPHYLL	outer phyllary length (mm) measured from phyllary base to tip
OPHYLW	outer phyllary width (mm) measured at widest point
OPHYLWTOE*	outer phyllary measured from widest point to tip (mm)
OPHYLV	outer phyllary number of veins
IPHYLL	inner phyllary length (mm) measured from phyllary base to tip
IPHYLW	inner phyllary width (mm) measured at widest point
IPHYLWTOE*	inner phyllary measured from widest point to tip (mm)
IPHYLV	inner phyllary—number of veins
RFLOR	number of ray florets per head
RSTRAPL	ray lamina length (mm) measured from top of corolla tube to tip of lamina
RSTRAPWD*	ray lamina width (mm) measured at widest point
RACHL*	ray floret cypsela length (mm) at anthesis stage
RPAPL	ray floret pappus length (mm)
DFLOR	number of disk florets per head
DCORL	disk corolla tube length (mm) measured from base to tip of corolla tube
DACHL	disk floret cypsela length (mm) at anthesis stage
DCORLIMB*	disk floret corolla limb length (mm)
DLOBL	disk corolla lobe length (mm)
DPAPL	disk pappus length (mm)
BRANCHD*#	distance between the first two branches at base of inflorescence

Assignment of the 160 specimens to the five a priori groups was based on morphological characters used in the literature to separate the taxa (Semple & Cook, 2006), on characters identified by us, and on provenance. Specimens were assigned a priori to *Solidago juliae* based mainly on geographical distribution, specifically presence on the Edwards Plateau and in Trans-Pecos, Texas, and adjacent Mexico; stems densely hairy with generally short hairs 0.5 ± 0.1 mm long and leaves grayish green due to high hair density. Following Semple and Cook (2006), the number of serrations in mid-stem leaves was used to identify *S. leavenworthii*; these plants came from the outer coastal plain of Georgia and Florida. Specimens placed a priori in *S. tortifolia* came from the outer coastal plain from South Carolina to Louisiana and had smaller involucres than *S. leavenworthii* following Semple and Cook (2006). Illustrations and the ranges of these three North American species are shown in Semple (2015, continuously updated).

MULTIVARIATE ANALYSES

Univariate and multivariate statistical analyses were performed using SYSTAT 10 for Windows (SPSS Inc., 2000, Chicago, Illinois, U.S.A.). Discriminant analysis is a well-known tool in taxonomy. It has been used successfully in *Solidago* (e.g., Heard & Semple, 1988; Semple et al., 1990; Cook et al., 2009; Semple et al., 2013, 2015) and in other Astereae (Owen et al., 2006). It was used in this study to assess the distinctiveness of members of the *S. chilensis* complex from the North American relatives. It allows testing of the usefulness of key traits in published floristic treatments (e.g., density of the paniculiform inflorescence; Cabrera, 1971). Also, it provides critical statistical information on the probabilities that an individual specimen belongs in each group included in the analysis. Correct placement a posteriori into the a priori group can occur but with a low probability. The number of such specimens with low probabilities of placement is an indication of the distinctiveness of pairs of taxa. The goal is to find traits that have low within-group variance but high between-group variance.

Discriminant analysis consists of a set of sub-analyses. First, a pairwise Pearson correlation matrix was generated to determine which traits were highly correlated. When pairs of traits were correlated with $|r| > 0.7$, one trait of the pair was dropped from further analyses. This is done on the assumption that highly correlated traits may be under the control of a single gene with pleiotropic effects.

Then, using only the less correlated characters, stepwise discriminant analysis was used to select a subset of characters that maximize the Mahalanobis distances separating the a priori groups in N-dimensional hyperspace; these selected characters were then used to run a complete discriminant analysis. The number of characters that can be used is one less than the smallest sample size in any a priori group. Characters used are selected based on the F-statistic of removal, with larger F-values indicating greater importance in separating a priori group centroids in N-dimension hyperspace. Next, classificatory discriminant analyses (linear and jackknife) were performed on the data matrix using traits identified by the stepwise analysis and a discriminant function to assign individual specimens to groups a posteriori. Included in these analyses were tests for equality of group centroids (Wilks' lambda, Pillai's trace, and Hotelling-Lawley trace) for testing null hypotheses and the determination of Geisser assignment probabilities of a posteriori placement of each specimen into each a priori group. Finally, a canonical discriminant analysis was used as a dimension-reduction technique to facilitate visualization of the results of the multidimensional analyses.

Initially, a subset of 110 South American specimens was analyzed to assess character variation and test hypotheses on the number of taxa. Then, a discriminant analysis was performed on the full 160-sample matrix in order to assess differences between North American and South American members of *Solidago* subsect. *Triplinerivae* and as a way of assessing the level of difference between the two South American a priori groups compared to the three North American species-level a priori groups.

CYTOLOGY

Mitotic chromosome numbers were determined from root tip cells of plants grown from field-collected cypselae at the University of Waterloo Department of Biology greenhouse following Owen et al. (2006). Counts were made from cells of freshly squashed root tips. Vouchers of all collections have been deposited in WAT with duplicates in LP.

RESULTS

The means, standard deviations, and ranges of the 50 traits scored on 160 specimens of *Solidago chilensis*, *S. juliae*, *S. leavenworthii*, *S. microglossa*, and *S. tortifolia* were calculated and are presented in Table 2.

PRELIMINARY DISCRIMINANT ANALYSES

Three preliminary discriminant analyses were performed to test support for hypothesized taxa in South America using only the 116 specimens from South America; naturalized collections of *Solidago chilensis* from Madeira Island and the West Indies were not included. The distribution of South American specimens included in the analysis is shown in Figure 1; ranges are based on Lopez Laphitz (2009). Details for all analyses were presented in Lopez Laphitz (2009). First, in order to test the usefulness of a published floristic key (Cabrera, 1971), a two a priori group analysis was performed: *S.* "*patagonica*" with lax inflorescence and *S. chilensis* with dense inflorescence. Although the two groups differed significantly overall (i.e., the null hypothesis that the two groups were not different was rejected; alpha < 0.05), a posteriori assignment probabilities of individual specimens were often low and misclassifications were frequent. In a second discriminant analysis, hair density was used to define two varietal level a priori groups: *S. chilensis* s.l. with a high hair density (variety *megapotamica* sensu Cabrera) and *S. chilensis* with no hairs (glabrous; variety *chilensis* sensu Cabrera). Again, although the two groups differed significantly and the null hypothesis was rejected, a posteriori placement of many individuals had low probabilities of correct placement and many specimens were misclassified. Third, based on our observations, the *S. chilensis* complex was tentatively split into two putative species-level taxa based on the mid-stem hair length: *S. chilensis* with short hairs (mean of 0.2 ± 0.1 mm long) and *S. microglossa* with long hairs (mean of 0.9 ± 0.15 mm long). In the classificatory discriminant analysis, correct a posteriori assignments occurred for more than 90% of the individuals included in the analysis for both of the putative taxa. Following these results, two species-level groups were tentatively recognized in the *S. chilensis* complex in South America: *S. chilensis* and *S. microglossa*. Mid- and upper stem hair densities versus hair lengths for the specimens of the two species are plotted in Figure 2.

FIVE A PRIORI GROUPS ANALYSIS: TWO SOUTH AMERICAN AND THREE RELATED NORTH AMERICAN TAXA

A discriminant analysis was performed including all 160 specimens to determine which characters were most useful in separating the five species-level a priori groups: two South American, *Solidago chilensis* and *S. microglossa*, and three North American, *S. juliae*, *S. leavenworthii*, and *S. tortifolia*. The eight characters with highest F-values selected in a stepwise discriminant analysis were included in a complete discriminant analysis: ISTPUBD (stem hairs, number of hairs counted on 1 mm on the inflorescence stem), ULSERAT (stem hairs, number of hairs counted on 1 mm on the upper vegetative stem), CAPW (width of the inflorescence measured at the widest point [cm]), OPHYLL (outer phyllary length [mm] measured from the phyllary base to tip), RFLOR (number of ray florets per head), BSTPUBD (stem hairs, number of hairs counted on 1 mm on the basal stem), MLWID (mid-stem leaf width [cm], as measured from widest point to blade apex), and IPHYLV (inner phyllary number of veins) (Tables 1, 2) in decreasing order of discriminatory power. The F-matrix based on Mahalanobis distances between group centroids and associated probabilities indicate strong support for the five taxa (Table 3). All three tests for equality of group centroids (Wilks' lambda, Pillai's trace, and Hotelling-Lawley trace) indicated that the groups overall were significantly different (Table 4), and the null hypothesis that all groups were really the same was rejected. The F-values between *S. juliae* and *S. tortifolia* were the lowest, while values between *S. chilensis* and *S. tortifolia* were the highest.

Geisser a posteriori assignment probabilities were determined for each of the 160 specimens, and a classification matrix and a jackknifed classification matrix for these specimens were generated (Tables 5 and 6, respectively).

In the classificatory analysis, eight of the nine specimens assigned a priori to *Solidago juliae* were assigned a posteriori to that taxon. Five of these specimens had Geisser assignment probabilities between 93% and 100%, while the other three had assignment probabilities between 78% and 84%. One specimen assigned a priori as *S. juliae* was misclassified and assigned a posteriori very weakly to *S. leavenworthii* with Geisser assignment probabilities split among *S. leavenworthii* (42%), *S. juliae* (42%), and *S. tortifolia* (16%).

Of the 15 specimens assigned a priori to *Solidago leavenworthii*, 10 were assigned a posteriori to that taxon. Nine of the specimens had Geisser assignment probabilities between 99% and 100%, and one specimen had an assignment probability of 76%. Five of the specimens assigned a priori to *S. leavenworthii* were misclassified with assignment probabilities between 0% and 41% to the a priori group. Two of the specimens were placed a posteriori in *S. chilensis* (68% and 57% probabilities, respectively), and one specimen was placed in *S. juliae* (62% probability).

Table 2. Mean ± standard deviation and range (minimum–maximum) by species level for the a priori groups for the 45 traits included in the stepwise discriminant analysis of 160 specimens of *Solidago* subsect. *Triplinerviae*: *S. chilensis* Meyen, *S. microglossa* DC., *S. juliae* G. L. Nesom, *S. leavenworthii* Torr. & A. Gray, and *S. tortifolia* Elliott. See Table 1 for trait descriptions. N refers to the number of specimens scored for each species.

Trait	*S. chilensis*	*S. microglossa*	*S. juliae*	*S. leavenworthii*	*S. tortifolia*
	N = 88	N = 31	N = 11	N = 15	N = 15
STEMHT (cm)	64.8 ± 32.7	81 ± 28.3	95.2 ± 38.2	84.1 ± 29.2	83.3 ± 35.2
	7–170	36–167	60–180	47–165	30–140
ISTPUBD	3.8 ± 3.1	8.9 ± 2.3	14.8 ± 2.9	9.31 ± 3.2	11.5 ± 2.25
	2–16	2–22	11–27	3–20	6–21
USTPUBD	2.8 ± 2.65	8 ± 2.7	14.5 ± 2.7	5.3 ± 3	11 ± 2.5
	0–0	1–17	8–22	0–13	5–18
MSTPUBD	1.8 ± 2.2	6.7 ± 2.4	12.15 ± 4	3.05 ± 3	10.6 ± 2.5
	0–0	0–13	0–20	0–12	6–18
BSTPUBD	0.8 ± 1.8	4.6 ± 2.3	5.4 ± 5.4	0.4 ± 0.8	5.5 ± 4
	0–0	0–13	0–12	0–4	0–14
ISTHLN (mm)	0.2 ± 0.1	0.8 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
	0.06–0.75	0.21–1.5	0.19–0.75	0.21–0.5	0.19–0.67
USTHLN (mm)	0.2 ± 0.1	0.9 ± 0.15	0.5 ± 0.1	0.25 ± 0	0.3 ± 0.1
	0.06–1.25	0.21–1.67	0.21–0.94	0–0.37	0.19–0.67
MSTHLN (mm)	0.2 ± 0.1	0.9 ± 0.3	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
	0.06–0.5	1.75–0.5	0.21–0.75	0–0.44	0.12–0.56
BSTHLN (mm)	0.1 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0
	0.06–0.75	0.21–1.25	0.12–0.5	0.06–0.5	0.12–0.44
MLLENG (mm)	61 ± 18	58.5 ± 18.2	50 ± 11.4	65.35 ± 19.8	40 ± 9.1
	3–130	22–105	27–88	26–103	25–65
MWID (mm)	6.6 ± 2.3	8.7 ± 3	7.2 ± 2.2	8 ± 1.9	5 ± 1.4
	0–32	4–19	4–17	4–13	2–8
MLWTOE (mm)	27.2 ± 9.1	27.1 ± 8.8	23.2 ± 5.5	31.7 ± 9.25	18.8 ± 4.5
	0–60	12–50	9–35	10–50	10–30
MSERAT	1.9 ± 2.4	2.7 ± 2	3.1 ± 4.5	7.2 ± 3.55	1.93 ± 0.8
	0–12	0–8	0–14	0–15	0–4
ULLENG (mm)	36.5 ± 12.3	30.4 ± 11	31 ± 10.5	37 ± 16.6	20.9 ± 8
	0.5–80	11–55	12–80	10–94	10–41
ULWID (mm)	3.95 ± 2.3	4.7 ± 1.7	4.6 ± 0.9	4.6 ± 1.7	3.1 ± 0.7
	0–27	1.5–10	2.5–9	2–10	1.5–5
ULWTOE (mm)	17.5 ± 6.3	15.6 ± 5.8	14.1 ± 3.5	18 ± 7.6	10.3 ± 3.95
	0–40	6–35	6–28	5–42	5–20
ULSERAT	0.5 ± 1.3	0.3 ± 0.6	0.9 ± 1.45	3.7 ± 2.8	0.7 ± 0.86
	0–10	0–5	0–6	0–13	0–4
ILLENG (mm)	18.65 ± 6.5	17.3 ± 5.5	18.05 ± 7.3	22.4 ± 8.5	12.2 ± 4.5
	4.4–45	6–40	8–40	5–43	4–30
ILWID (mm)	2.2 ± 1.5	2.8 ± 1	2.9 ± 1.2	3 ± 1.1	1.95 ± 0.5
	0.5–2.21	1–15	1–7	1–6	1–4
ILWTOE (mm)	8.95 ± 3.45	8.7 ± 3.3	8.6 ± 3.6	11.2 ± 4.5	6.1 ± 2.3
	1–25	3–27	4–20	2–29	2.5–14
ILSERAT	0.05 ± 0.3	0.01 ± 0.07	0.3 ± 0.8	1.15 ± 1.3	0.15 ± 0.4
	0–5	0–1	0–4	0–7	0–2
CAPBRANG	27.9 ± 9.85	29.8 ± 14.5	34.5 ± 5.3	27.9 ± 7.9	38.2 ± 6.1
	1–60	15–90	30–40	9–40	30–50
CAPW (cm)	12.0 ± 6.5	13.7 ± 6.5	21.7 ± 8.4	13.3 ± 6.7	15.4 ± 3.4
	1–30	3–27	11–37	2.5–29	9–21
CAPL (cm)	8.7 ± 6.6	8.8 ± 4.8	8.3 ± 1.7	6.8 ± 5.0	9.4 ± 3.6
	2–35	2–18	5.5–1	1.5–20	3–15
LONGBR (cm)	5.5 ± 4.5	5.1 ± 3	7.3 ± 3.4	6.3 ± 6.2	6 ± 2.25
	2–35	1–12	3.5–12	1.5–20	2–10
INVOLHT (mm)	4.4 ± 0.5	4.4 ± 0.4	3.4 ± 0.25	4.3 ± 0.6	3.1 ± 0.2
	3–7	3–6	4.1–2.6	2.9–6	2.6–4
OPHYLL (mm)	1.8 ± 0.2	1.7 ± 0.2	2.6 ± 4.1	1.6 ± 0.3	1.1 ± 0.1
	1.1–2.7	0.17–2.6	0.8–3	1–2.6	0.7–1.6

Table 2. Continued.

Trait	*S. chilensis*	*S. microglossa*	*S. juliae*	*S. leavenworthii*	*S. tortifolia*
OPHYLW (mm)	0.55 ± 0.1	0.5 ± 0.05	0.5 ± 0.2	0.6 ± 0.1	0.3 ± 0
	0.3–1.6	0.3–0.7	0.3–1.3	0.4–0.8	0.2–0.6
OPHYLWTOE (mm)	1.3 ± 0.2	1.3 ± 0.15	1 ± 0.2	1.2 ± 0.3	0.7 ± 0.2
	0.5–2.2	0.8–1.8	0.4–1.3	0.6–2.1	0.3–1.3
OPHYLV	1.1 ± 0.2	1.05 ± 0.2	1 ± 0	1 ± 0	0 ± 1
	1–3	1–3	1–1	1–1	1–1
IPHYLL (mm)	3.8 ± 0.4	3.8 ± 0.35	2.8 ± 0.2	3.5 ± 0.5	2.6 ± 0.3
	2.4–5.1	0.29–4.8	2.3–3.5	2.6–5	1.9–3.6
IPHYLW (mm)	0.6 ± 0.1	0.6 ± 0.1	0.55 ± 0.05	0.7 ± 0.1	0.49 ± 0.1
	0.3–1	0.3–0.8	0.4–0.7	0.5–1	0.3–0.8
IPHYLWTOE (mm)	1.9 ± 0.2	1.8 ± 0.2	1.4 ± 0.1	1.7 ± 0.3	1.3 ± 0.2
	0.9–2.6	1–2.4	0.8–1.7	0.1–2.9	0.9–2
IPHYLV	1.9 ± 0.7	2.3 ± 0.7	1.7 ± 0.6	1.7 ± 0.8	1.25 ± 0.4
	1–7	1–3	1–3	1–3	1–3
DFLOR	13.5 ± 3.1	15.1 ± 3.6	10.4 ± 1.8	11.7 ± 4.5	4.75 ± 1.2
	7–29	4–28	6–18	7–29	2–8
RSTRAPL (mm)	2.9 ± 7.8	1.9 ± 0.3	1.3 ± 0.25	1.9 ± 0.4	1.35 ± 0.35
	0.4–3.1	1–2.9	0.7–1.8	1–3	0.7–4
RSTRAPWD (mm)	0.5 ± 0.7	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
	0.1–0.9	0.1–0.7	0.1–0.6	0.2–0.8	0.1–1.5
RACHBL (mm)	1.1 ± 3.7	0.8 ± 0.6	0.8 ± 0.2	0.8 ± 0.2	0.6 ± 0.2
	0.3–3.3	0.4–4	0.5–1.3	0–1.5	0.4–1.2
RPAPL (mm)	3.4 ± 0.5	3.1 ± 0.6	2.2 ± 0.45	2.95 ± 0.9	2.2 ± 0.2
	2.3–4.5	0.37–4.3	1–3	0.32–4.3	1.6–2.8
#DFLOR	9.2 ± 3.2	8 ± 1.85	5.8 ± 1	8.05 ± 5	3.6 ± 0.9
	2–25	2–30	2–7	4–30	1–6
DCORL (mm)	3.7 ± 0.4	3.6 ± 0.3	3.2 ± 0.2	3.8 ± 0.4	2.8 ± 0.3
	2.4–4.9	2.7–4.6	2.5–4	2.6–4.7	0.3–3.8
DACHBL (mm)	0.8 ± 0.2	0.8 ± 0.1	0.85 ± 0.2	0.8 ± 0.2	0.6 ± 0.2
	0.1–2.6	0.5–1.2	0.5–1.3	0.5–1.2	0.4–1.3
DCORLIMB (mm)	2.6 ± 0.4	2.5 ± 0.3	2 ± 0.15	2.5 ± 0.3	1.9 ± 0.2
	1.5–4.6	1.4–3.5	1.5–2.5	1.7–3.5	1.3–2.5
DLOBL (mm)	2 ± 0.3	1.05 ± 0.2	1.1 ± 0.3	1 ± 0.1	0.8 ± 0.3
	0.12–3.9	0.6–2.3	0.4–2.7	0.7–1.5	0.4–1.9
DPAPL (mm)	3.6 ± 0.5	3.4 ± 0.4	2.6 ± 0.3	3.5 ± 0.5	2.2 ± 0.5
	0.8–5.1	2.3–4.4	2–3.1	2.1–4.4	0.5–3

All 14 specimens assigned a priori to *Solidago tortifolia* were assigned a posteriori to that taxon. Eleven of the specimens were classified with probabilities of 92%–100%; one was classified with only 63% probability to *S. tortifolia* and 37% to *S. juliae*.

Of the 88 specimens assigned a priori to *Solidago chilensis*, 77 were assigned a posteriori to that taxon, with 60 specimens having Geisser assignment probabilities between 91% and 100% and 17 specimens having assignment probabilities between 62% and 88% (two specimens with less than 75% from Jujuy, Argentina). Seven of the misclassified specimens were assigned to *S. microglossa* with Geisser assignment probabilities between 51% and 98% (from Jujuy, Río Muerto [Chaco], Salta, and Tucuman, Argentina, and Cochabamba, Licoma, and Santa Cruz, Bolivia), and three were assigned to *S. leavenworthii* with probabilities between 99% and 100% (from Jujuy, Salta, and San Luis States in Argentina). One was placed in *S. juliae* with 77% probability (from Santa Cruz, Bolivia).

Of the 30 specimens assigned a priori to *Solidago microglossa*, 28 were assigned a posteriori to that taxa. Nineteen of the specimens were assigned with 90%–100% probability to *S. microglossa*; nine were assigned with 51%–89% probability. Two specimens were assigned a posteriori to *S. chilensis*, with 60% and 86% probability (from Formosa, Argentina, and Central Department, Paraguay, respectively).

A canonical analysis was performed, and the canonical variant scores on the first and second and on the first and third canonical axes (CAN 1, CAN 2, and CAN 3) were plotted for the five putative taxa

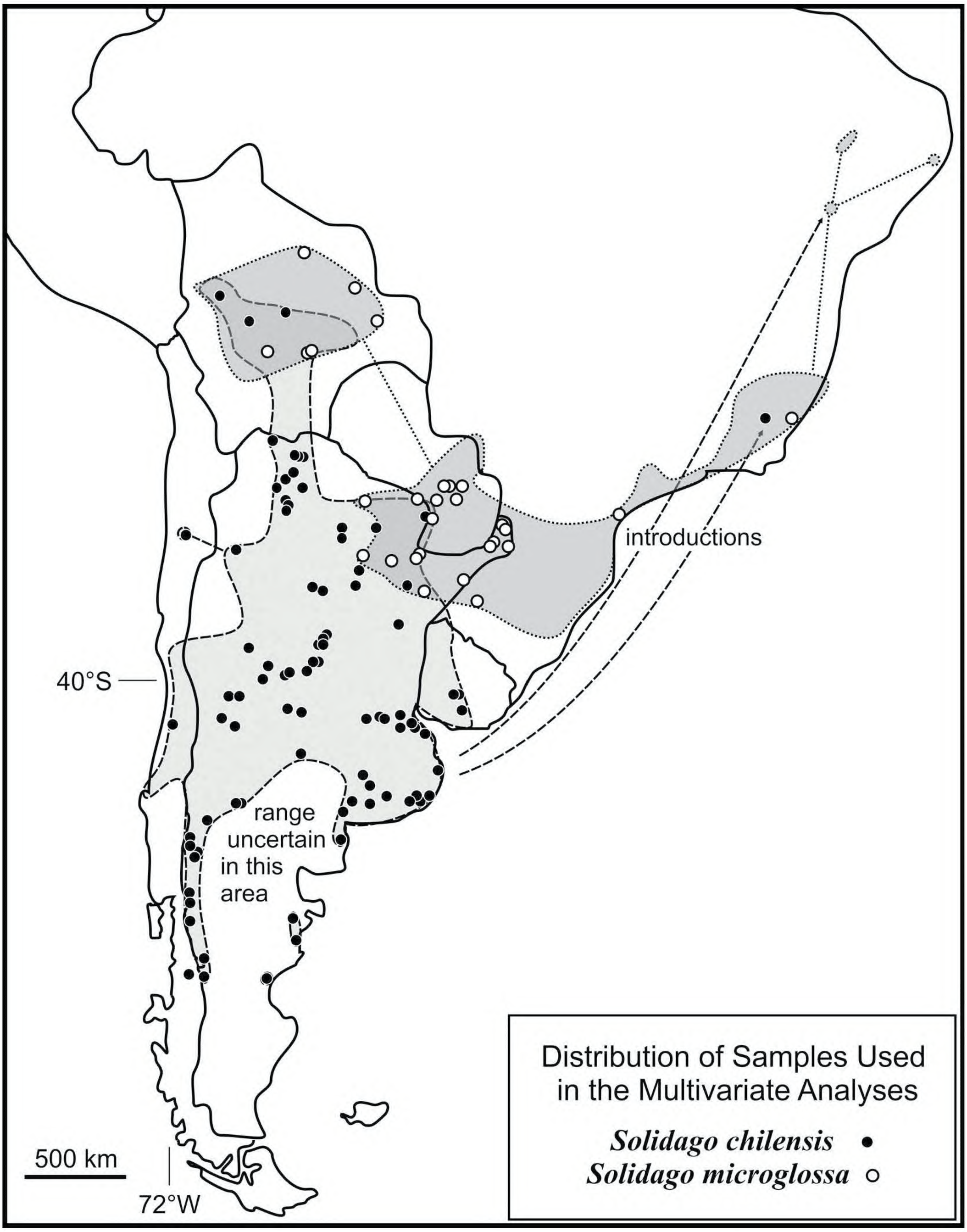


Figure 1. Distribution in South America of 116 specimens of *Solidago chilensis* (black dots) and 33 specimens of *S. microglossa* (white dots). The location of a specimen from Madeira Island, Portugal, is not shown. Shaded areas indicate the ranges of distribution based on Lopez Laphitz (2009).

studied (Fig. 2; 95% confidence ellipses included). The eigenvalue for the first canonical variate (3.121) accounted for 87.5% of the variation, the second (1.560) for 11.2%, and the third (0.666) for an additional 1.3%.

CHROMOSOME COUNTS

Chromosome numbers were determined for 27 plants grown from field-collected cypselae at the University of Waterloo Department of Biology green-

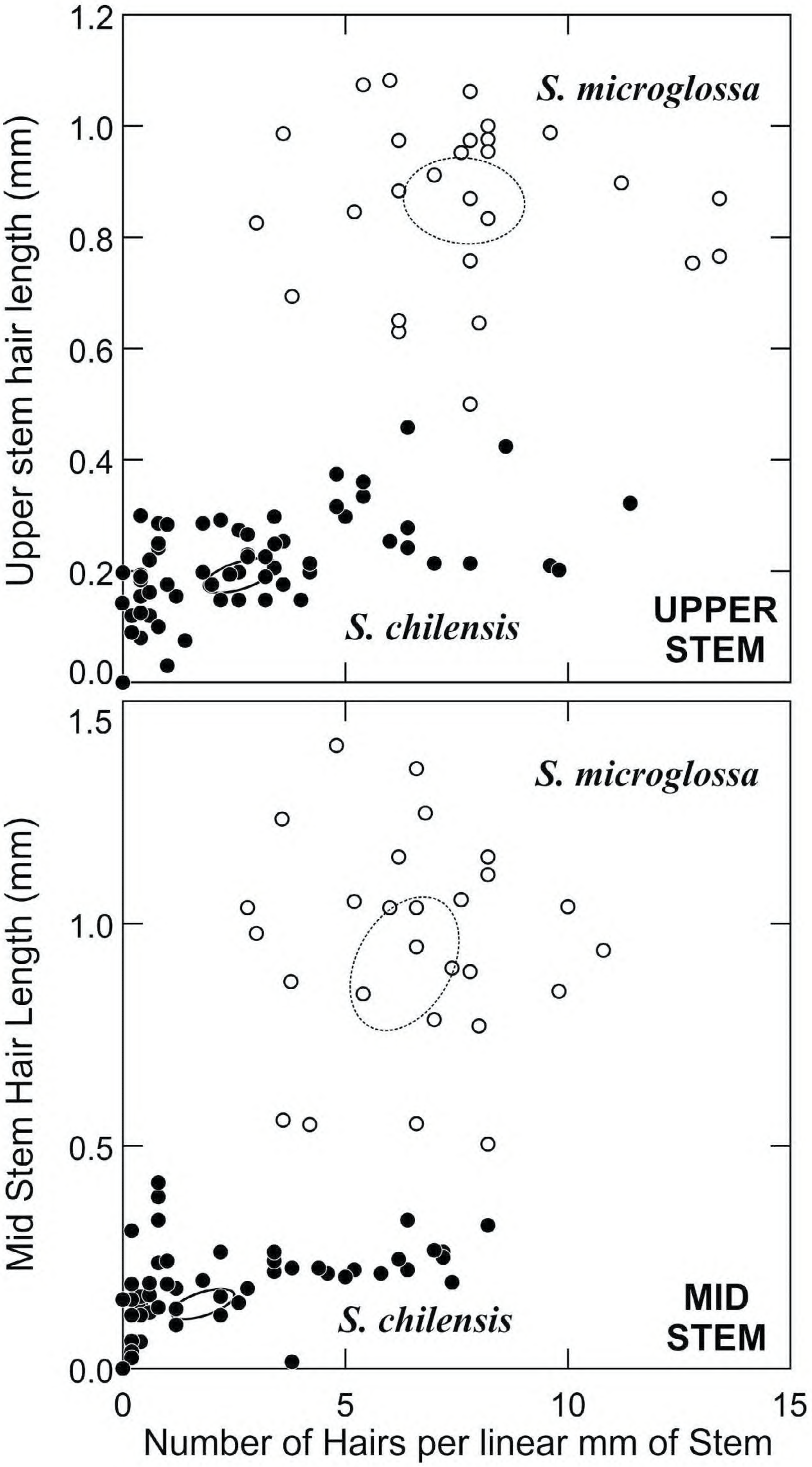


Figure 2. Scatterplots of length of hairs (mm) versus the number of hairs per mm of mid- and upper stems for specimens of *Solidago chilensis* (dots) and *S. microglossa* (circles).

Table 3. F-statistics $F_{0.05}$ and associated null hypothesis probabilities among the five putative taxa; $P < 0.0000$ in all cases; *Solidago chilensis* Meyen, *S. microglossa* DC., *S. juliae* G. L. Nesom, *S. leavenworthii* Torr. & A. Gray, and *S. tortifolia* Elliott.

	S. microglossa	*S. chilensis*	*S. juliae*	*S. leavenworthii*
S. chilensis	17.633			
S. juliae	14.191	27.430		
S. leavenworthii	17.704	16.336	12.818	
S. tortifolia	29.805	40.429	4.446	16.694

Table 4. Multivariate statistics and F-approximations for the Wilks' lambda, Pillai's trace, and Hotelling-Lawley trace tests from the discriminant analysis of the five species-level groups: *Solidago chilensis* Meyen, *S. microglossa* DC., *S. juliae* G. L. Nesom, *S. leavenworthii* Torr. & A. Gray, and *S. tortifolia* Elliott.

	Value	F-values	DF	*P* tail
Wilks' lambda	0.060	18.094	32, 532	0.0000
Pillai's trace	1.716	13.799	32, 588	0.0000
Hotelling-Lawley trace	5.022	22.364	32, 570	0.0000

house (Appendix 1). All counts were diploid $2n = 18$; 21 for *Solidago chilensis* from Argentina and Chile and six counts for *S. microglossa* from Argentina.

Discussion

Solidago in South America

Based on univariate and discriminant analyses and observations on plants in the field and greenhouse, two native species of *Solidago* subsect. *Triplinerviae* should be recognized in South America: *S. chilensis* and *S. microglossa*. The nomenclature was chosen after observations were made on type specimens and available high-resolution photographs of the type specimens (Lopez Laphitz, 2009). The character found most reliable in separating the two taxa was stem hair length. Other traits used in the literature to separate species or varieties of South American goldenrods did not yield groups that were as statistically well supported or had high numbers of specimens with low probabilities of placement into the a priori groups.

Observations of plants grown in the greenhouse clearly showed that hair length was an inheritable character and a trait little influenced by environmental conditions. The selection pressures favoring short versus long hairs and differences in hair densities are unknown. Hair density is variable in *Solidago* subsect. *Triplinerviae* and the variation in hair density in *S. chilensis* is similar to that seen in *S. lepida* of North America (Semple et al., 2013). The complete lack of hairs on the stem is an extreme condition in *S. chilensis* as is clear in Figure 2. Published keys in Cabrera (1974) and Sancho and Viera Barreto (2014) separating glabrous (*S. chilensis* var. *chilensis*) and pubescent (*S. chilensis* var. *megapotamica*) races of a single species, *S. chilensis* s.l., do not account for the large number of specimens of *S. chilensis*, as delimited here with statistical support, that have some hairs on the midstem or just the upper stem and in the inflorescence. Hair length is critical in separating the two species *S. chilensis* and *S. microglossa* (synonym, *S. chilensis* var. *megapotamica*) used in conjunction with several other traits (e.g., mid-stem leaf width, numbers of ray florets; see Key to Species below).

In contrast, the inflorescence density character used in the published floristic keys (Cabrera, 1971; Sancho & Espinar, 2003) could be affected by different environmental factors (Jakobs et al., 2004) and is based on a misapplication of the name *Solidago patagonica*. The type specimen of *S. patagonica* Phil. (*Cox s.n.*, SGO, digital image!) was determined to be a member of *S. chilensis*, and the name belongs in synonymy. Sancho and Viera Barreto (2014) noted that the inflorescence density difference really applies to a non-native escaped cultivar collection of *S. virgaurea*, native to Europe. Lopez Laphitz (2009) first noted this fact.

A member of *Solidago* subsect. *Junceae* was reported from South America for the first time and identified as *S. missouriensis*-like in Lopez Laphitz (2009) and subsequently described as *S. argentinensis* Lopez Laphitz & Semple (Lopez Laphitz et al., 2011). Comparison of the South American specimens with multiple species of North American *Solidago* subsect. *Junceae* confirmed that South American plants fit well within the description of the subsection in Semple and Cook (2006), with some variations such as phyllaries sometimes with multiple veins and often minutely glandular. A multivariate morphometric study of North and South American members of subsection *Junceae* was conducted by Lopez Laphitz et al. (2011). Sancho and Viera Barreto (2014) included *S. argentinensis* in their floristic treatment.

Solidago subsect. *Triplinerviae* in South America and related taxa in North America

The discriminant analysis including *Solidago chilensis* and *S. microglossa* from South America and *S. juliae*, *S. leavenworthii*, and *S. tortifolia* from North America provided strong support for recognizing all five a priori groups. The null hypothesis was rejected and five species-level groups accepted. The South American *Solidago* specimens were as differ-

Table 5. Classification matrix obtained by classificatory discriminant analysis of the five putative taxa: *Solidago chilensis* Meyen, *S. microglossa* DC., *S. juliae* G. L. Nesom, *S. leavenworthii* Torr. & A. Gray, and *S. tortifolia* Elliott. Rows show number of specimens in an a priori group assigned to each a posteriori group and the percent of specimens in an a priori assigned a posteriori to the a priori group.

	A posteriori					
A priori	*S. microglossa*	*S. chilensis*	*S. juliae*	*S. leavenworthii*	*S. tortifolia*	% correct
S. microglossa	28	2	0	0	0	93%
S. chilensis	7	77	1	3	0	88%
S. juliae	0	0	8	1	0	89%
S. leavenworthii	1	3	1	10	0	67%
S. tortifolia	0	0	0	0	14	100%
Totals/Mean	36	82	10	14	14	88%

ent from the three North American taxa as these three species were different from each other. Although the analysis showed five well-separated groups, classificatory analyses showed that some specimens of *S. leavenworthii*, *S. chilensis*, and *S. microglossa* had weak support for placement a posteriori in their a priori group or were misclassified. For instance, 11 specimens identified a priori as *S. chilensis* were misclassified; three were placed a posteriori in *S. leavenworthii*, seven in *S. microglossa*, and one in *S. juliae*. These specimens were re-examined, and they showed ambivalent morphological characteristics (problematic specimens), such as longer than usual hairs for *S. chilensis* in contrast to the a priori classification that defined *S. chilensis* as short-haired plants. Furthermore, these problematic specimens were restricted to two geographical areas: (1) the northwestern portion of the range in South America, including Bolivia and northern Argentina, and (2) northeastern Argentina in the provinces of Corrientes and Formosa. Because these ambivalent morphological traits were restricted to two geographic areas, three hypotheses were suggested to account for the a posteriori assignments of *S. chilensis* specimens to other species. First, Bolivia and adjacent areas are postulated as the probable starting point for the location of the original introduction from North America (possibly from northern Mexico) with subsequent unknown evolutionary pressures resulting in differentiation into two well-defined, mostly allopatric taxa occupying different habitats. Second, both geographic areas are tentatively suggested as zones of potential interspecific hybridization due to more recently occurring sympatry; this applies to the *S. chilensis* specimens assigned a posteriori to *S. microglossa*. Lopez Laphitz (2009) and Gonçalves Silva et al. (2009) provided evidence of human-aided, long-distance dispersal of *S. chilensis* into equatorial Brazil, the West Indies, and the Madeira Islands off the coast of Africa. Human-aided dispersal of *S. microglossa* in South America also seems likely. Third, local variation in some traits in *S. chilensis* resulted in several pockets of somewhat divergent populations that happened to be more similar to North American species; the specimens were correctly placed a priori in *S. chilensis*, but were atypical for the species. The a posteriori assignments to *S. juliae* and *S. leavenworthii* on technical traits should not be taken as evidence that these two North American species are present in South America because the specimens had the traits used to assign them a priori into the *S. chilensis* group.

Table 6. Jackknifed classification matrix obtained by classificatory discriminant analysis of the five putative species: *Solidago chilensis* Meyen, *S. microglossa* DC., *S. juliae* G. L. Nesom, *S. leavenworthii* Torr. & A. Gray, and *S. tortifolia* Elliott. Rows show number of specimens in an a priori group assigned to each a posteriori group and the percent of specimens in each a priori group assigned a posteriori to the a priori group.

Group	A posteriori					
A priori	*S. microglossa*	*S. chilensis*	*S. juliae*	*S. leavenworthii*	*S. tortifolia*	% correct
S. microglossa	28	2	0	0	0	93%
S. chilensis	8	76	1	3	0	86%
S. juliae	0	0	6	1	2	67%
S. leavenworthii	1	3	1	10	0	67%
S. tortifolia	0	0	1	0	13	100%
Totals/Mean	37	81	9	14	15	88%

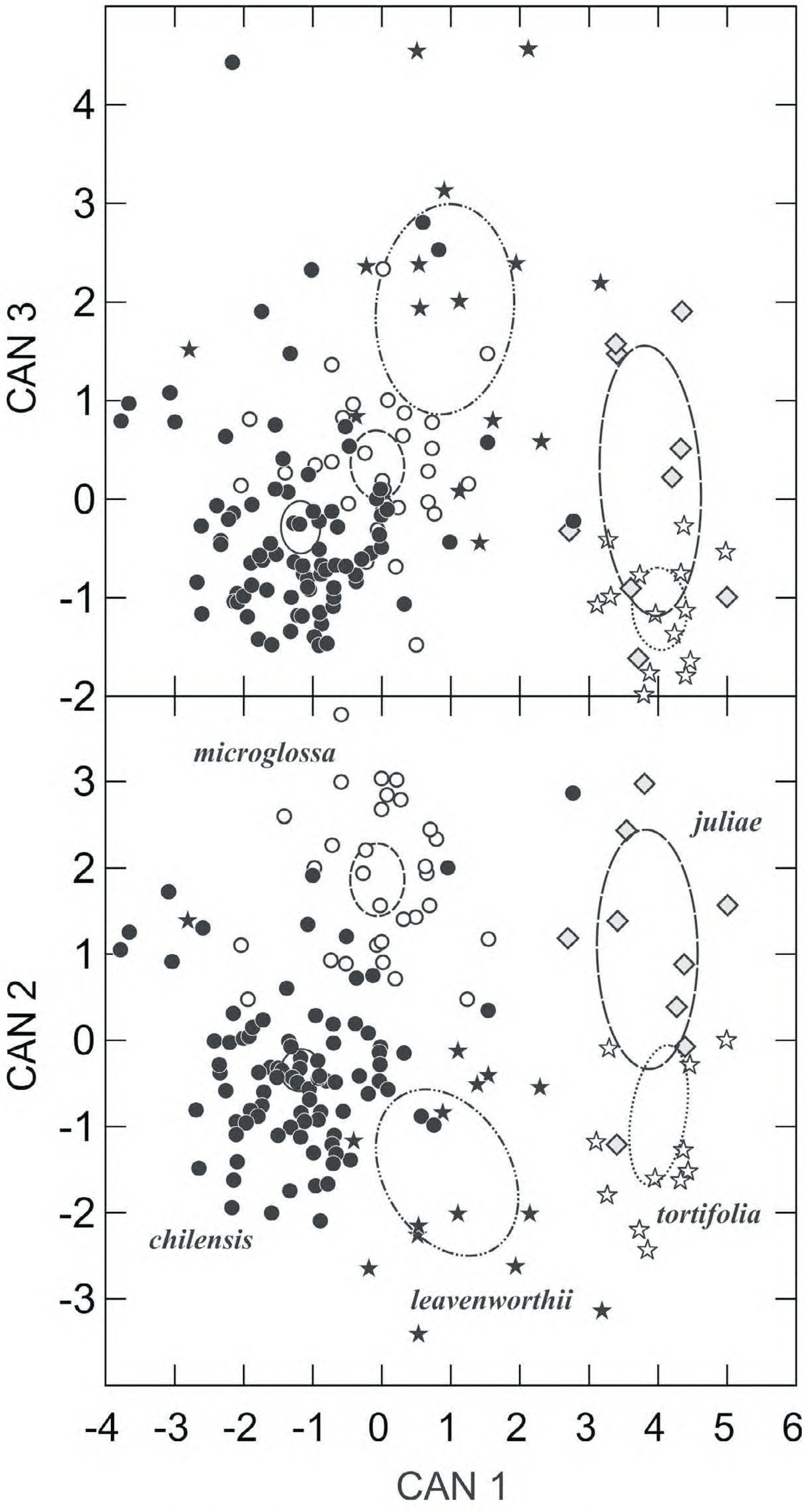


Figure 3. Scatterplots of the first and second (bottom) and the first and third (top) canonical scores (CAN 1, CAN 2, CAN 3) for five species-level groups with 95% confidence intervals indicated for each group by broken lines. *Solidago chilensis* is represented by solid dots and solid line ellipses, *S. juliae* by shaded diamonds and short broken line ellipses, *S. leavenworthii* by solid stars and dash-dot ellipses, *S. microglossa* by open circles and long dash ellipses, and *S. tortifolia* by open stars and dotted line ellipses.

The origins of *Solidago* in South America have not been studied in detail. However, the presence of North American taxa in central and southern South America could be a result of island hopping down the cordillera via similar habitats that no longer exist in Central America and northern South America or the result of long-distance transport of the fruit propagules attached to the feathers of migrating birds. Alan Graham (pers. comm.) suggested the latter is much more likely as there is little evidence for the existence of intermediate, temporary habitats similar to those found in northern Mexico and Bolivia today. Evidence of recent long-distance dispersal of *S. chilensis* to locations in the Northern Hemisphere, e.g., Puerto Rico in the West Indies (Lopez Laphitz, 2009) and Madeira Island off the coast of Africa (Gonçalvez Silva et al., 2009), clearly shows that long distance is not a barrier to migration and colonization by members of *Solidago* subsect. *Triplinerviae*. Also, the North American *S. altissima*, a relative of *S. chilensis* in subsection *Triplinerviae*, has naturalized in eastern China, Taiwan, and Japan (Chen & Semple, 2011).

Results also suggest that the three morphologically similar species from southern North America included in the discriminant analyses and the two species from South America possibly shared a common ancestor in the Pleistocene, and all five species are relatively recently evolved by the same processes of founder effect, isolation, and adaptation. The times of divergences are speculative, but advances and retreats of glaciers would have provided a strong stimulus for expansion and contraction of ranges in North America and Canada. *Solidago altiplanities*, native to the southern High Plains of Texas and Oklahoma, is also morphologically similar to the five species analyzed in this study and may have diverged from a common ancestor during earlier glacial peak and contraction events. Of note is the fact that only in South America did the plesiomorphic short, dense stem pubescence of the putative ancestral taxon evolve into both nearly glabrous and longer-haired conditions found separately in the two South American species. In North America, the three species remained short haired and generally densely so. Only in *S. leavenworthii*, the only known polyploid species in the complex, did North American plants approach the glabrous/glabrate condition seen in the extreme in some plants in *S. chilensis* in South America. *Solidago altissima* also has similar hairy stems and may be related to these species, although it has morphological affinities (Semple et al., 2015) with other species of *Solidago* subsect. *Triplinerviae* as well, making its phylogenetic position uncertain within the subsection.

Interspecific hybridization is common among *Solidago* species in North America (Semple et al., 1999), and it should not be excluded as a possible explanation for the presence of specimens with ambivalent identity in South America. For instance, observations on this study of somewhat longer hairs in specimens classified a posteriori as *S. chilensis* showed possible evidence of hybridization with longer haired *S. microglossa*. However, this hypothesis was not tested with experimental crosses between *S. chilensis* and *S. microglossa*. Semple and Semple (1977) noted that there was a greater variability in morphological characteristics of hybrids than expected from parental species. Therefore, similar variability might be seen in the wild hybrid swarms including some of the misclassified specimens. Further work is needed to document the presence of *S. chilensis* × *S. microglossa* hybrids in Bolivia and northern Argentina.

DISTRIBUTION AND CYTOGEOGRAPHY OF *SOLIDAGO* SUBSECT. *TRIPLINERVIAE* IN SOUTH AMERICA

In total, 32 diploid ($2n = 18$) chromosome number determinations for populations of *Solidago chilensis* and *S. microglossa* have been reported either here (Appendix 1) or previously (Covas & Schnack, 1946, 1947; Turner et al., 1979; Hunzinker et al., 1989; Dematteis et al., 2007). The ranges of distributions of *S. chilensis* and *S. microglossa* and the distribution of cytological samples are shown in Figure 4. The new counts are in agreement with previous reports listed under various species and variety names. Although some obviously larger-headed individuals from South America were included in the multivariate analyses, the variation cannot be explained by polyploidy. In contrast, in North America, polyploidy is common in most species of *Solidago* subsect. *Triplinerviae* (Semple et al., 1984; Semple & Cook, 2006). In other parts of the world, it is typically the polyploids of species of subsection *Triplinerviae* that have successfully naturalized, e.g., *S. gigantea* Aiton (Schlaepfer et al., 2008a, 2008b, 2010) and *S. altissima* (Chen & Semple, 2011). Of note, the putative close relatives of *S. chilensis* and *S. microglossa* in North America (*S. juliae*, *S. tortifolia*, and *S. altiplanities*) are all known only at the diploid level, but sample sizes are very small. Additional work on the cytogeography of the South American species is needed to sample all parts of the range in addition to those indicated in Figure 4.

TAXONOMIC TREATMENT OF *SOLIDAGO* IN SOUTH AMERICA

I. Solidago L., Sp. Pl. 2: 878. 1753. TYPE: *Solidago virgaurea* L. (lectotype, designated by Britton & A. Brown, Ill. Fl. N. US, ed. 2(3): 380. 1913.

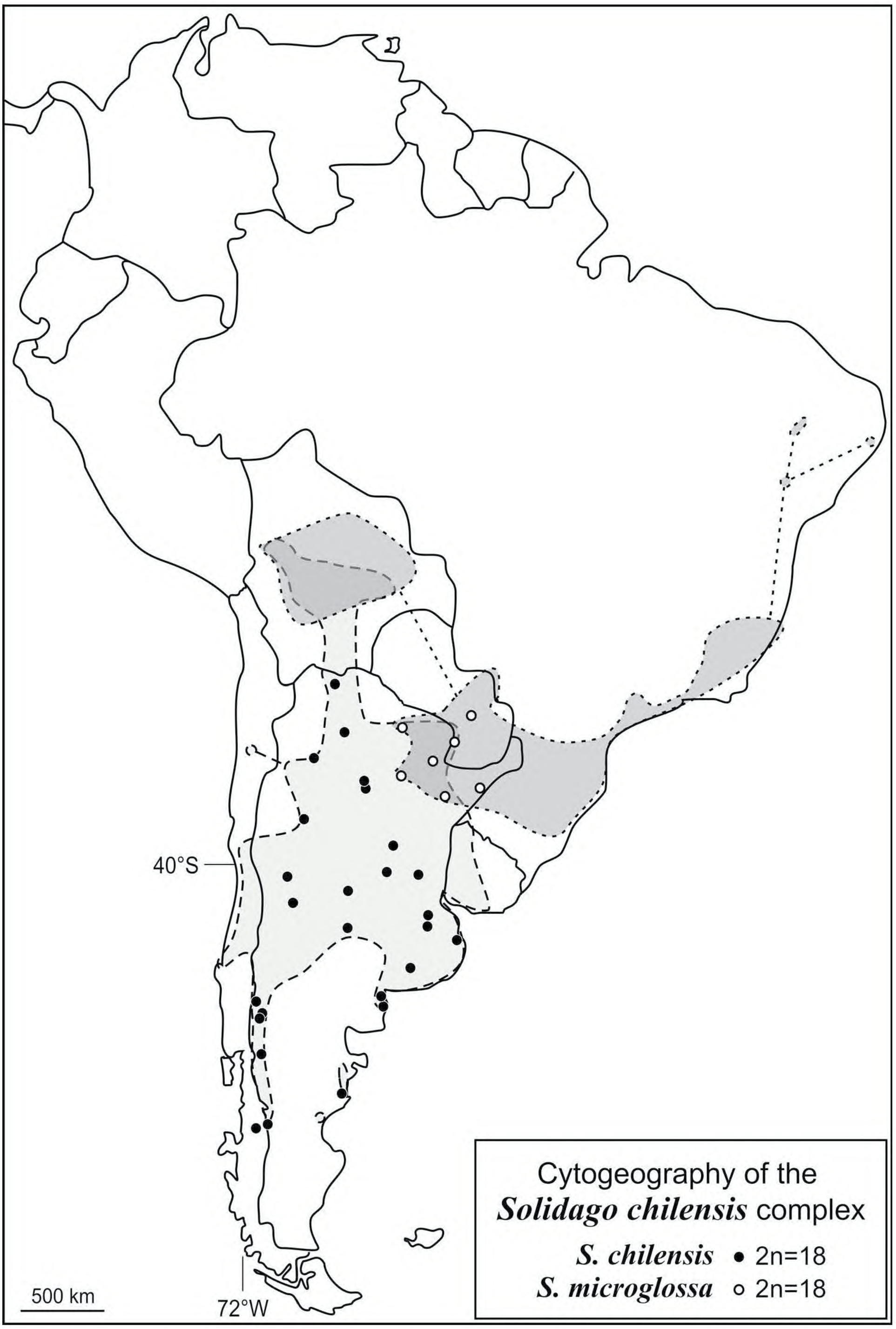


Figure 4. Cytogeographic map of *Solidago chilensis* (black dots) and *S. microglossa* (white dots) in South America based on chromosome counts reported herein and from the literature.

KEY TO *SOLIDAGO* IN SOUTH AMERICA

1. Basal rosettes usually present at time of flowering; rosette and basal stem leaves the largest, pairs of small lateral branch leaves often present in axes of upper stem leaves; stems proximally glabrous, sparsely strigose distally; involucres 3.5–5.5 mm high, phyllaries ovate, linear-ovate, or oblong ... *S. argentinensis* Lopez Laphitz & Semple (2).
1′. Basal rosettes not present at time of flowering; lower part of the mid-stem leaves largest or lowest stem leaves largest and stems pubescent; stem pubescence to glabrate; involucres 3.5–10 mm high, phyllaries broadly to narrowly lanceolate.
   2. Inflorescence lax; involucres 6–10 mm; basal leaves the largest; stem leaves relatively few; not triple-nerved, stems glabrate; not native ..... *S. virgaurea* L. (1).
   2′. Inflorescence congested; involucres 3.5–5.5 mm high at anthesis; lower part of the mid-stem leaves the largest; stem leaves many, triple-nerved; stems glabrate, canescent or short villose; native.
      3. Stem hairs short, 0.1–0.4 mm long; stem pubescent to glabrate ..... *S. chilensis* Meyen (3).
      3′. Stem hairs 0.4–1.2 mm long; stems hispid-villose ..... *S. microglossa* DC. (4).

Ia. Solidago subsect. **Solidago.**

Leaves basal, often present in rosettes at flowering; proximal-most cauline leaves petiolate to attenuate-subpetiolate, sometimes present at flowering, largest, distal reduced. Heads in racemiform, paniculiform arrays, sometimes corymbiform, wand- to club-shaped, branches ascending. Phyllaries usually not striate, glabrous to strigose, eglandular to sparsely glandular, lanceolate. Pappus bristles usually in 2 series, the inner series longest, somewhat to moderately clavate.

Included species. (Ca. 11 native to Eurasia and arctic and alpine North America) *Solidago dahurica* (Kitag.) Kitag. ex Juz., *S. decurrens* Lour., *S. kurilensis* Juz., *S. leiocarpa* DC., *S. litoralis* Savi, *S. minutissima* (Makino) Kitam., *S. multiradiata* Aiton, *S. nipponica* Semple, *S. pacifica* Juz., *S. spithamaea* M. A. Curtis ex A. Gray, and *S. virgaurea*.

1. Solidago virgaurea L., Sp. Pl. 880. 1753. TYPE: "Habitat in Europe pascuis siccis," *Herb. Linnaeus 998.15* (lectotype, designated by Garbari & Cecchi in Jarvis & Turland, 1998: 368, LINN 998.15!).

Syntypes. Solidago 3, *Clifford 409* (BM), Solidago 3a, *Clifford 409* (BM), Solidago 3b, fol. A, *Clifford 409*, Solidago 3b, fol. B, *Clifford 409* (BM), Solidago 3e, *Clifford 409* (BM); Herb. Burser XV: 66 (UPS).

Herbaceous perennials from short rhizomes; stems erect, 13.5–110 cm tall, glabrate below to sparsely to densely strigose distally. Leaves basal and proximal obovate to oblanceolate, often present at flowering, in most of the cases the biggest on the plant, petiolate, 4.5–21.1 × 0.9–4.2 cm, blades lanceolate to oblanceolate, abaxial and adaxial faces glabrous or sparsely strigose, abaxial veins sometimes densely strigose, margins serrate, ciliate; mid-stem leaves lanceolate to narrowly lanceolate, sessile, bases tapering, 3.5–11.2 cm, serrations 0 to 14; distal lanceolate to linear-lanceolate, reduced upward, 2–9.3 × 0.4–2.4 cm, serrations 0 to 9. Heads racemiform to thyrsiform-paniculiform arrays, congested and few-headed in short plants to lax in taller plants with longer branches and peduncles, branches ascending, sparsely to densely strigose. Involucres campanulate, 4.8–8 mm high at flowering, larger with age; phyllaries in 3 or 4 imbricate series, those of outer series ovate to lanceolate, middle lanceolate, 2.3–5.4 × 0.6–1.5 mm, apex tapering, with inner phyllaries oblanceolate to linear-lanceolate. Ray florets 1 to 10; laminae yellow, 2.9–7 × 0.5–2.1 mm; disk florets 1 to 29, averaging 13; corollas 4.1–6.9 mm, lobes 0.35–1.8 mm. Cypselae obconic to linear obconic, very sparsely to densely strigose over the entire body, body length at anthesis, 0.8–2.0 mm, to 3 mm at maturity; longest pappus bristles 3.6–6.4 mm. Chromosome count $2n = 18$. (Description based on European collections.)

Phenology. *Solidago virgaurea* has been observed as flowering in March in South America.

Habitat and distribution. *Solidago virgaurea* is known from one collection, *Cabrera 5015* (LP), from Rio Negro, Argentina. The species is native mainly to Europe and to Morocco and Pakistan, India, and Nepal (and possibly adjacent Tibet in China) in the mountains.

Ib. Solidago subsect. **Junceae** (Rydb.) G. L. Nesom, Phytologia 75: 11. 1993. *Solidago* [sp. group] *Junceae* Rydb., Fl. Plains N. Amer. 792. 1932. *Solidago* ser. *Junceae* (Rydb.) G. L. Nesom, Phytologia 75: 9. 1993. TYPE: *Solidago juncea* Aiton.

Leaves basal, often present in rosettes at flowering, proximal-most cauline petiolate to attenuate-subpetiolate, sometimes present at flowering, largest; cauline sometimes 3-nerved (with 2 prominent lateral nerves), distal usually subtending fascicles of small linear leaves of lateral branches, 2 usually much larger than others. Heads in thyrsiform-paniculiform arrays, wand- to club-shaped or sometimes secund and cone-shaped, proximal branches ascending to

spreading; phyllaries usually not striate, glabrous to strigose, eglandular to sparsely glandular. Pappus bristles usually in 2 series, inner series weakly, if at all, clavate (modified from Semple & Cook, 2006).

Included species. (Nine, North America, northern Mexico; South America, Argentina, Bolivia.) *Solidago argentinensis* Lopez Laphiz & Semple, *S. confinis* A. Gray, *S. durangensis* G. L. Nesom, *S. gattingeri* Chapm. ex A. Gray, *S. guiradonis* A. Gray, *S. juncea* Aiton, *S. missouriensis*, *S. pinetorum* Small, and *S. spectabilis* (D. C. Eaton) A. Gray.

2. Solidago argentinensis Lopez Laphitz & Semple, Novon 21(2): 223. 2011. TYPE: Argentina. Mendoza: Dpto. San Rafael, Rio Salado Superior entre, 29 Jan. 1966, *Ruiz Leal 24529* (holotype, LP!).

Herbaceous perennials from short to long rhizomes; stems erect, 18–50 cm tall, glabrate at base, with increasing hair density distally, strigulose. Basal cauline leaves sometimes present at flowering and relatively large; mid- to distal cauline leaves much reduced distally; often subtending clusters (fascicles) of small lateral branch leaves; lower stem leaves petiolate, 20–80 mm including petiole, 3–14 mm wide, margins serrations 0 to 7; mid-stem leaves sessile, rapidly reduced upward, oblanceolate to lanceolate, 25–65 × 3–12 mm, margins serrations 0 to 5; upper and inflorescence stem leaves sessile, reduced, lanceolate to linear, 12–58 × 0.5–5 mm. Heads paniculiform (pyramidal), nodding or secund, 5–21 × 2–6 cm; involucres 3.5–5.5 mm high; phyllaries in 3 or 4 series, those of the outer series ovate, with the inner phyllaries linear-ovate to oblong. Ray florets 6 to 19, strap 1–3 × 0.2–0.7 mm; disk florets 5 to 19, corollas 3–4.8 mm, lobes 0.6–1.5 mm. Cypsellae obconic, 0.5–1.2 mm; pappus bristles 3.2–5 mm. Chromosome number unknown.

Phenology. *Solidago argentinensis* has been collected in flower from January to early March.

Habitat and distribution. *Solidago argentinensis* is known from Patagonia in Argentina and to the north (Chubut, Mendoza, Neuquen, and Rio Negro Provinces). It has been collected from Juncal, Chile, and Valle de Cochabamba, Bolivia.

Ic. Solidago subsect. **Triplinerviae** (Torr. & A. Gray) G. L. Nesom, Phytologia 75(1): 8. 1993. Basionym: *Solidago* [unranked] *Triplineriae* Torr. & A. Gray, Fl. N. Amer. 2(2): 222. 1842. TYPE: *Solidago canadensis* L. (lectotype, designated by Nesom, 1993: 8).

Leaves with basal rosettes absent; proximal cauline leaves nearly always withering by flowering, sessile, proximal, most not as large as lower mid-stem leaves and sometimes distal, 3-nerved (2 lateral nerves prominent, sometimes obscurely so and only on proximal leaves). Heads in thyrsiform-paniculiform arrays, usually congested cone-shaped (pyramidal), secund, sometimes leafy arrays (club-shaped in *Solidago elongata*), proximal branches often arching, secund, branches sometimes shorter than subtending leaves; phyllaries usually not striate, sometimes minutely stipitate-glandular. Pappus bristles usually in 2 series, the series sometimes similar, inner usually weakly if at all clavate, rarely moderately so (modified from Semple & Cook, 2006).

Included species. (Ca. 16, 11 in the flora, from North America, South America; introduced in Eurasia, Africa, Oceania). *Solidago altissima*, *S. altiplanities*, *S. brendiae*, *S. canadensis*, *S. chilensis*, *S. elongata*, *S. fallax*, *S. gigantea*, *S. gypsophila* G. L. Nesom, *S. juliae*, *S. leavenworthii*, *S. lepida*, *S. microglossa*, *S. muelleri* Standl., *S. pringlei* Fernald, *S. rupestris* Raf., *S. shortii* Torr. & A. Gray, and *S. tortifolia*.

3. Solidago chilensis Meyen, Reise Erde 1: 311. 1834. TYPE: Chile. "in campus chilesibus," de Chile y Pampas (in sched. label), s.d., *Née s.n.* (holotype, B†; neotype, designated here, G-DC!, photo G-DC010427_1!).

Solidago linearifolia DC., Prodr. [de Candolle] 5: 341. 1836. *Solidago microglossa* DC. var. *linearifolia* (DC.) Baker in Martius, Fl. Bras. 6(3): 10. 1882. TYPE: Chile. "in campus Chilensibus," s.d., *Née s.n.* (holotype, G-DC!).

Solidago coquimbana Phil., Linnaea 33: 138. 1864–1865. TYPE: Chile. Cordera: Cordillera de Thapel, Jan. 1860, *Volikmann s.n.* (holotype, SGO not seen, photo!).

Solidago laxiflora Phil., Anales Univ. Chile 87: 429. 1894. TYPE: Chile. S. Francisco del Monte, 1880, *Martio s.n.* (holotype, SGO not seen, photo! [in sched., "typus" by R. Acevedo de V., 1957]).

Solidago valdiviana Phil., Anales. Univ. Chile 87: 431. 1894. TYPE: Chile. Valdivi: San Juan, aestati 1887, *Philippi s.n.* (holotype, SGO not seen, photo! [in sched., "typus," C. Muñez P., 1944]).

Solidago parviflora Phil., Anales Univ. Chile 87: 432. 1894. non *Solidago parviflora* Raf., Ann. Nat. 14. 1820. TYPE: Chile. Pirihuaico, Feb. 1887, *Otto s.n.* (holotype, SGO not seen, photo! [in sched., "typus," R. Acevedo de V., 1957]).

Herbaceous perennials from short to long rhizomes, 7–170 cm; stems erect, glabrate proximally, glabrate to densely canescent distally, hairs 0.05–0.3 mm. Leaves with the proximal ones oblanceolate, twisted, nearly always withering well before flower-

ing; mid-stem leaves usually persisting, in most cases the biggest, sessile, blades linear to oblanceolate, 13–130 × 2–32 cm, with 0 to 12 serrations, abaxial and adaxial faces glabrous or sparsely strigose; distal leaves linear-lanceolate, smaller than proximal, 10–80 × 1–27 mm, with 0 to 10 serrations; inflorescence leaves linear to lanceolate, 4.4–45 × 0.5–18 mm with 0 to 5 serrations. Heads 20 to 200+; in secund lax to dense pyramidal paniculiform arrays, 5–30 × 1–35 cm, branches spreading recurved, second; involucres campanulate, 3.5–5.5 m high; phyllaries in 3 or 4 series, those of outer series ovate, 1.1–2.7 × 0.3–1.6 mm, with inner phyllaries oblanceolate, 0.29–5.1 × 0.3–1 mm, 1 to 3 nerves. Ray florets 7 to 21; laminae yellow, 0.4–3.1 × 0.1–0.9 mm; disk florets 2 to 30; corollas 2.4–4.9 mm, lobes 0.12–3.9 mm. Cypselae body, 0.6–1.5 at anthesis, to 3.3 mm at maturity, sparsely to densely strigose; longest pappus bristles 1.9–5.1 mm. Chromosome count $2n = 18$.

Phenology. *Solidago chilensis* has been observed to flower mainly between December and the end of April and once in late August.

Habitat and distribution. *Solidago chilensis* is known from Argentina (all provinces except for Misiones), Chile, Bolivia, and Uruguay. This species has been found as an introduction in Brazil; Madeira Island, Portugal; Peru; and Puerto Rico. It ranges from latitudes 14°–47°S and longitudes 56°–73°W and elevations from 7 to 2600 m. It is common on roadsides, open grassy plains, and wet soil along streams and springs and is found on sandy soils.

Discussion. The type for *Solidago chilensis* was destroyed at B in 1943, and no Meyen collections remain at B (Voss, pers. comm.). With no original material found, the name is neotypified upon the type of its earliest synonym *S. linearifolia* DC., upon a Née collection.

Solidago chilensis is distinguished by its usually glabrate aspect or with stem hairs mostly 0.05–0.3 mm long; plants from Bolivia tend to be more densely canescent. It is mainly from cold and temperate climates in habitats such as along the roads and in fields and close to water sources (ponds, rivers). In the Patagonian Monte the species forms patches along roads, while in pampas (flatlands) and jungle it reaches further distances from the road within the local flora.

4. Solidago microglossa DC., Prodr. [de Candolle] 5: 332. 1836. *Aster polyglossus* var. *microglossus* (DC.) Kuntze, Revis. Gen. Pl. 3[3]: 131. 1898. TYPE: Brazil. S.d., *Bacle s.n.* (holotype, GDC!, photo sent by G GDC01425_1!, P photo at MO ex Krukoff!).

Solidago microglossa var. *megapotamica* DC., Prodr. [de Candolle) 5: 332. 1836. *Solidago chilensis* Meyen var. *megapotamica* (DC.) Cabrera, Darwiniana 16: 409. 1970. TYPE: Brazil. Prov. Rio Grande: in "hb. Mus. reg. Par. à Mus. imp. Bras. miss. sub n. 846, 854, et 857 [protologue]." *Gaudichaud 854* (holotype, G-DC not seen, photo!, P photo at MO ex Krukoff!).

Solidago marginella DC., Prodr. [de Candolle] 5: 332. 1836. TYPE: Brazil. S. loc., s.d., *Bacle s.n.* (holotype, G-DC not seen, photo!).

Solidago polyglossa DC., Prodr. [de Candolle] 5: 332. 1836. *Aster polyglossa* (DC.) Kuntze, Rev. Gen. 1: 318. 1891. TYPE: Brazil. Rio Grande "v.s. in h. Mus. reg. Par. à Mus. imp. Bras. sub n. 860 missa." s.d., *Gaudichard 860* (holotype, G-DC, photo!, fragm., P photo at MO ex Krukoff!).

Solidago odora Hook. & Arn. var. *scabra* Hook & Arn., Companion Bot. Mag. 2: 45. 1836. TYPE: Uruguay. Monte Video, *Tweedy s.n.* (holotype, K!).

Solidago microglossa var. *macrophylla* Niederl., Bol. Mens. Mus. Prod. Argent. 3, no. 29: 186. 1890, nom. nud.

Solidago bonaerensis Larrañaga, Escritos Damaso Antonio Larranaga 2: 255 [Pub. Inst. Hist. Geog. Uruguay]. 1923, nom. nud.

Plants 36–167 cm tall, stems decumbent from branching rhizome, densely pubescent, canescent. Leaves with the proximal ones oblanceolate, twisted, nearly withering well before flowering; proximal persisting, in most cases the biggest ones of the plant, sessile; proximal blades lanceolate, 22–105 × 5–19 mm, with 0 to 8 serrations, abaxial and adaxial faces pubescent; distal linear to lanceolate, 11–55 × 2–10 mm with 0 to 5 serrations; inflorescence leaves linear to lanceolate, 6–40 × 1–15 mm with 0 or 1 serration. Heads many, in secund pyramidal paniculiform arrays, branches recurved, second. Involucre campanulate, 3.5–6 cm high; phyllaries in 3 or 4 series, those of outer series ovate, 0.17–2.6 × 0.3–0.7 mm, with inner phyllaries oblanceolate, 2.7–4.8 × 0.3–1 mm, 1 to 3 nerves. Ray florets 4 to 28; laminae, yellow, 1–2.9 × 0.1–0.6 mm; disk florets 2 to 14; corollas 2.7–4.5 mm, lobes 0.6–2.3 mm. Cypselae body length at anthesis, 0.4–1.4 mm, strigose; longest pappus bristles 1.8–4.4 mm. Chromosome count $2n = 18$.

Phenology. *Solidago microglossa* has been observed in flower from December until the end of April, with an extension of the flowering period due to favorable weather conditions until mid-spring (October).

Habitat and distribution. *Solidago microglossa* is known from Bolivia, Paraguay, Brazil, and northern Argentina (Misiones, Corrientes, Formosa, Chaco). It has been collected from savannas, open grassy areas,

and riversides as well as from secondary vegetation along roadsides and wet grasslands. Collections range from latitudes 7°–39°S, longitudes 34°–67°W, and elevations from 200 to 2740 m.

Discussion. *Solidago microglossa* is characterized by moderately to densely pubescent stems (hairs 0.4–1.2 mm long) and leaves. The species is found mainly in subtropical warm climates, occurring along roads and close to water sources (ponds, rivers). It is inserted as part of the densely local vegetation, such as grasslands and jungle; in Brazil, it is distributed along the coast.

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Appendix 1. Chromosome number determinations for *Solidago chilensis* and *S. microglossa* were made from root-tip cells taken from greenhouse-grown plants at the University of Waterloo, Department of Biology, following Owen et al. (2006). Plants were grown from cypselae collected in Argentina and Chile. All vouchers were collected by R. M. Lopez Laphitz and L. A. Becker (*LL & B*) and have been deposited in WAT with duplicates in LP.

Solidago chilensis Meyen., $2n = 18$.

ARGENTINA. **Buenos Aires:** Saldungaray, a. Al lado de la RP 72, *LL & B 4*. **Catamarca:** RN 38 hacia San Fernando del Valle de Catamarca, *LL & B 27*. **Chubut:** Gaiman, camino La Angostura hacia Dolavon, *LL & B 7*; Casi Epuyen, RN 40 desde Esquel, *LL & B 12*. **Cordoba:** Vickuna Mackenna, RN 35, *LL & B 20*. **Entre Rios:** RN 127 entre San Jaime de la Frontera y los Conquistadores, *LL & B 50*. **La Pampa:** RN 35, empalme con RP 10, *LL & B 19*. **La Rioja:** RN 141 y 79, *LL & B 26*. **Mendoza:** Monte Coman, RP 171, *LL & B 23*. **Neuquen:** RN 40, Km. 2069 casi limite con Rio Negro–Neuquen, *LL & B 14*; Junin de los Andes, *LL & B 16*; RN 40, camino de Junin de los Andes a Zapala, *LL & B 17*. **Rio Negro:** limite Rio Negro y Neuquen, RN 40 pasando Dique Alicurá 20 km hacia Neuquen, *LL & B 15*. **Salta:** RN 16 entre El Tunal y El Galpon, *LL & B 39*. **Santa Cruz:** Los Antiguos, *LL & B 9*. **Santa Fe:** Venado Tuerto, RN 8, *LL & B 1*; RP 95 pasando Pozo Borrado, *LL & B 34*; RN 127, *LL & B 51*. **Santiago del Estero:** RN 34 a 15 km sur del empalme con RP 92, *LL & B 31*; RN 64, a. A 3 km de Santiago del Estero, *LL & B 30*. CHILE. **Region XI:** ruta 265 (Camino Austral), a. Al lado de El Fachinal, *LL & B 10*.

Solidago microglossa DC., $2n = 18$.

ARGENTINA. **Chaco:** RN 16, a. A 10 km O de Roque Saenz Peña, *LL & B 36*; RN 16, saliendo del Puente General Belgrano, *LL & B 42*. **Corrientes:** Santo Tomé, *LL & B 47*; RN 123 entre RP 119 y RP 29, *LL & B 48*. **Formosa:** RN 11 llegando a Formosa Capital, *LL & B 43*; RN 81 pasando Estanislao del Campo, *LL & B 41*.

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☐ Synonyms based on different types are placed in separate paragraphs, each beginning with the basionym, followed by other combinations (if appropriate), and citation of the type.

☐ A Latin or English diagnosis for each new taxon is provided, immediately following the new taxon's name and type designation and immediately preceding the English or Spanish description of the new taxon. The *International Code of Nomenclature for Algae, Fungi and Plants* (Glossary) defines a diagnosis of a taxon as "a statement of that which in the opinion of its author distinguishes the taxon from other taxa." The journal's suggested style for the Latin diagnosis of a new species might include "Haec species . . . affinis [similis], sed ab ea [eo] . . . distinguitur [differ]," or in place of "Haec species" beginning with "Species nova" or equivalent wording. Suggested style for the English diagnosis of a new species might include "This [new] species is related [similar] to . . . , but is distinguished [differs] from it by . . ." or equivalent wording. Reference to diagnostic characters elsewhere in the text (abstract, discussion, key, etc.) is not acceptable in place of the formal diagnosis.

☐ For species with infraspecific taxa: Description and discussion are composite (incorporating all infraspecific taxa) and parallel with other species descriptions. Descriptions of infraspecific taxa are parallel with one another (in the same species). All synonyms are listed under the appropriate infraspecific taxon.

☐ Descriptions: Descriptions are parallel, within a given rank. All measurements are metric. Hyphens are used for parenthetical extremes: "peduncle (8.2–) 14.3–28.0(–31.9) cm long," unless intermediate values are not expected: ovary with (2)4(6) locules. Length × width are given in the following manner: lamina 36.4–82.8 × 9.1–16.8 cm.

☐ When relevant, nomina nuda, misapplied names, and superfluous names are included in the discussion following the description, but are not part of the formal synonymy.

7. Citation of Types

☐ Exclamation points are used for specimens examined, and types not seen are indicated as such (e.g., MO!, US not seen).

☐ Lectotype designations are included together with an indication of where they were designated [author, year, page number, and herbarium of deposition; e.g., *C. J. W. Schiede 159* (lectotype, designated by Stevens [2000: 256], P!; isotypes. . .)]. This reference is listed in the Literature Cited. If the author of the paper submitted is making the lectotypification, the phrase "designated here" is used.

8. Tables

☐ Create tables using the Microsoft Word "Table" feature.

☐ Tables are neat, double-spaced, and clearly presented.

☐ Captions are typed double-spaced as paragraphs at the tops of the tables.

☐ Each table starts on a separate page.

9. Abbreviations

☐ Periods are used after all abbreviations (which are minimized) except metric measures, compass directions, and herbarium designations.

☐ When dates are given as part of collection information, three-letter month abbreviations are used, except for months with four letters, which are spelled out in full.

☐ States are not abbreviated, and cities are spelled out. [St., as in St. Louis, is acceptable.]

☐ Periodicals are abbreviated according to *B-P-H* (*Botanico-Periodicum-Huntianum*) and to *B-P-H/S* (*Botanico-Periodicum-Huntianum/Supplementum*).

☐ Authors' names are abbreviated according to Brummit & Powell's *Authors of Plant Names*.

☐ Book titles are abbreviated according to *Taxonomic Literature, edition 2*, but with initial letters capitalized. Book titles are spelled out in the Literature Cited.

☐ If an item does not appear in *B-P-H* or *TL-2*, or if these references are not available, its title is fully spelled out.

☐ Herbaria are abbreviated according to the most recent edition of *Index Herbariorum*.

☐ Abbreviated forms are not used for references in the text, except when citing the names of plants. If it is necessary to cite a particular page in the text, the form Smith (1998: 12) is used.

10. Specimens Examined

☐ If many specimens were examined, those cited in the text are limited to ca. 12 manuscript pages.

☐ If there are a large number, an index to specimens examined is placed at the end of the paper, following the Literature Cited. It is arranged alphabetically by collector, followed by collection number, followed by the number of the taxon in the text. Names (including initial(s)) of first and second collector are provided, "et al." if three or more.

☐ Specimens are cited in the text as follows: *Additional specimens examined* (or *Selected specimens examined*). MEXICO. **Oaxaca:** Sierra San Pedro Nolesco, Talea, 12°37′N, 85°14′W, 950–1100 m, 3 Feb. 1987 (fl), *Jergensen 865* (BM, G, K, US). [Dates and reproductive status are optional but are omitted from longer lists.] Countries are run together in the same paragraph, e.g., COUNTRY A. **Major political division:** . . . COUNTRY B. **Major political division:** . . . Separate paragraphs are used for major continental regions within major political divisions.

11. Specimen Vouchers and Genetic Sequences

☐ If the paper presents original data, associated herbarium vouchers are cited. [Vouchers for seed and/or other collections should be included where pertinent. Dependent on the paper, reference to the original wild source may be required.] Vouchers are also cited from common names, and uses are taken from specimen labels.

☐ Herbarium vouchers state the collector and number, herbarium in which the voucher is located, and a clear annotation that the material represents the voucher for the study in question.

☐ Nucleic acid or protein sequences corresponding to equal or greater than 50 nucleotides are entered into an appropriate data bank, e.g., GenBank/EMBL. The accession numbers are provided before publication. [Long sequences (exceeding two pages) will not be routinely published.]

☐ Data sequences and tree files should be provided in Nexus format or alternatively submitted to the TreeBASE web site (<www.treebase.org>).

☐ Author accepts responsibility for establishing the accuracy of information provided.

12. Keys

☐ Keys are clear and have been checked carefully for consistency with the descriptions. Leads of each couplet are parallel.

☐ Dichotomous keys are indented.

☐ Infraspecific taxa are keyed separately, not in species keys.

☐ Authorities are listed for every species appearing in a key.

13. Literature Cited

☐ The Literature Cited contains full citations of all references cited in the text.

☐ All entries in the Literature Cited are cited in the text.

☐ Spelling of author(s) name(s) and years of publication have been double-checked.

☐ All entries have been verified against original sources, especially journal titles, accents, diacritical marks, and spelling in languages other than English.

☐ Periodicals are listed as follows: Author's last name, initial(s). Year. Full title of article. Journal abbreviated as in *B-P-H/S*. Volume: pages. No parenthetical part numbers after volume numbers are given unless each part is paginated separately.

☐ For more than one author, this style is followed: Author's last name, initial(s), second author's initial(s), last name & third author's initial(s), last name.

☐ Books appear as follows: author's last name, initial(s). Year. Full Unabbreviated Title (edited by Editor), 3rd ed., Vol. 2. Publisher, City of Publication.

☐ For an article within a larger work, this style is followed: Author(s). Year. Name of the article. Pp. 00–00 *in* Name of the editor(s), Full Title of Larger Work. Publisher, City of Publication.

☐ Citations of work "in prep.," unpublished theses and dissertations, and similar references to inaccessible sources have been eliminated or kept to a minimum. They are not necessarily included in the Literature Cited.

14. Illustrations

☐ Electronic figures are labeled with the first author's last name, the first four letters of the taxon, and "...Figure1.tif", "...Figure2.tif", etc. The file extension clearly indicates what type of file it is.

☐ Scale bars appear on illustrations, photographs, and maps.

☐ Magnifications/reductions are not indicated in captions.

☐ Electronic scans may be pre-sized to fit either column width (2-5/8 in. or ca. 68 mm) or full-page width (5-1/2 in. or ca. 140 mm). [Maximum size for printed illustrations is 5-1/2 × 8-1/4 in.]

☐ Figures are numbered in Arabic numerals in the order of their citation in the text. Parts of figures are labeled with capital letters.

☐ Text in figures is in Times New Roman.

☐ Photographs are prepared according to the printer's requirements. [See Guide to Digital Art, Allen Press through the Publication Submission link on the opening page of <http://www.mbgpress.info/>.]

☐ Figures are grouped into composite plates when possible; edges of photographs are abutted.

☐ Edges of figures are squared.

☐ Maps include reference to latitude and longitude and are bounded by a fine border.

☐ Scanning electron micrographs are free of conspicuous charging.

☐ Axes on graphs are all labeled.

☐ Captions provide all explanatory text. Captions are separate from other text, one paragraph for each group of figures, and following the style in current issues of the *Annals*.

☐ Symbols on maps are legible, and reduction has been taken into consideration.

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